6 October 2006 | $10

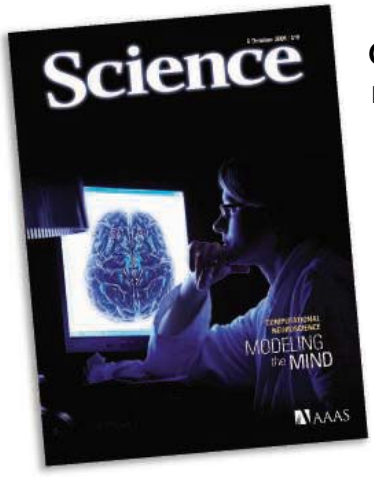
Science

COMPUTATIONAL NEUROSCIENCE

MODELING the MIND

AAAS

Volume 314, Issue 5796



COVER

Neuroscientists increasingly rely on the use of computers and simulations, because the systems they are studying are usually too complex for all of the data to be collected. It is thus a challenge to focus on the conceptually relevant parameters rather than simply trying to model reality by adding more and more details. See the special section on computational neuroscience beginning on page 75.

Image: Kelly Buckheit Krause (Photos: Rob Melnychu/Getty; Matthias Kulka/Corbis)

DEPARTMENTS

EDITORIAL

SPECIAL SECTION

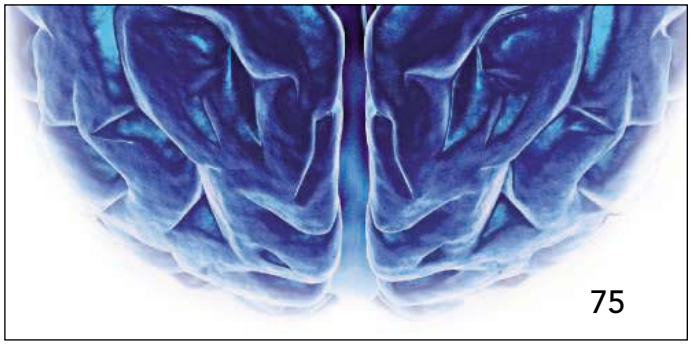
Modeling the Mind

INTRODUCTION

NEWS

REVIEWS

>> Editorial p. 17; Book Reviews pp. 57, 58, 59, and 60
For related online material, see p. 11 or go to www.sciencemag.org/sciext/compneuro/

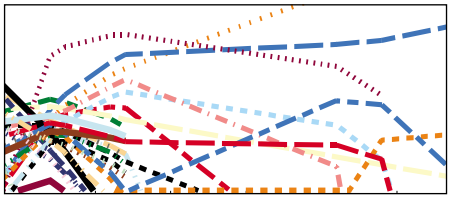


NEWS OF THE WEEK

NEWS FOCUS

CONTENTS continued >>

Science



SCIENCE EXPRESS

www.sciencexpress.org

GEOCHEMISTRY

Solar Nebula Heterogeneity in p-Process Samarium and Neodymium Isotopes

R. Andreasen and M. Sharma

Isotope ratios in primitive meteorites vary and differ from Earth's measured value, implying patchiness in the early solar nebula originating from two stellar sources.

>> News story p. 36

10.1126/science.1131708

GEOCHEMISTRY

Barium Isotopes in Chondritic Meteorites: Implications for Planetary Reservoir Models

M. C. Ranen and S. B. Jacobsen

Barium isotope variations in primitive meteorites suggest that they originated in a patch of the protosolar nebula that was enriched in supernova-derived material.

>> News story p. 36

10.1126/science.1132595

ECOLOGY

From Plant Traits to Plant Communities: a Statistical Mechanistic Approach to Biodiversity

B. Shipley, D. Vile, É. Garnier

A quantitative model based on plant traits can predict with more than 90 percent success the changes in species abundance that follow abandonment of agricultural sites in southern France.

10.1126/science.1131344

NEUROSCIENCE

Diminishing Reciprocal Fairness by Disrupting the Right Prefrontal Cortex

D. Knoch, A. Pascual-Leone, K. Meyer, V. Treyer, E. Fehr

Inhibition of a high-level brain region reduces an individual's ability to suppress selfish desires and produce generous acts.

10.1126/science.1129156

LETTERS

BOOKS *ET AL.*

POLICY FORUM

PERSPECTIVES

ESSAY

TECHNICAL COMMENT ABSTRACTS

PHYSICS

BREVIA

VIROLOGY

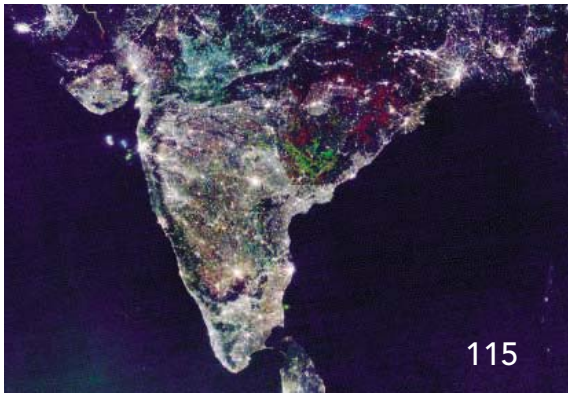
RESEARCH ARTICLES

ASTRONOMY

PALEONTOLOGY

CONTENTS continued **>>**

REPORTS



CREDIT: DISTRIBUTED BY NATIONAL GEOPHYSICAL DATA CENTER

AAAS
ADVANCING SCIENCE, SERVING SOCIETY

SCIENCE (ISSN 0036-8075) is published weekly on Friday, except the last week in December, by the American Association for the Advancement of Science, 1200 New York Avenue, NW, Washington, DC 20005. Periodicals Mail postage (publication No. 484460) paid at Washington, DC, and additional mailing offices. Domestic individual membership and subscription (51 issues): $139 ($74 allocated to subscription). Domestic institutional subscription (51 issues): $650; Foreign postage extra: Mexico, Caribbean (surface mail) $55; other countries (air assist delivery) $85. First class, airmail, student, and emeritus rates on request. Canadian rates with GST available upon request, GST #1254 88122. Publications Mail Agreement Number 1069624. **Printed in the U.S.A.**

Change of address: Allow 4 weeks, giving old and new addresses and 8-digit account number. **Postmaster:** Send change of address to AAAS, P.O. Box 96178, Washington, DC 20090–6178. **Single-copy sales:** $10.00 current issue, $15.00 back issue prepaid includes surface postage; bulk rates on request. The identification code for *Science* is 0036-8075. *Science* is indexed in the *Reader's Guide to Periodical Literature* and in several specialized indexes.

CONTENTS continued **>>**

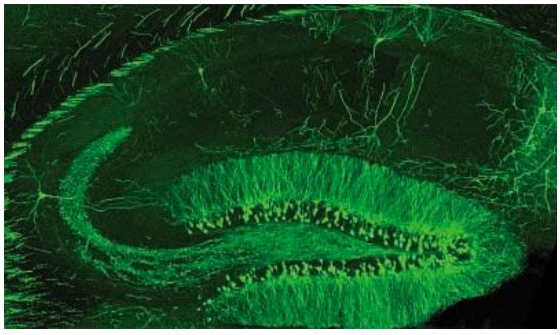
Setting New Heights

Elevate your research with Bio-Rad's wide range of high-quality protein standards for electrophoresis and blotting applications.

- Recombinant protein standards offer 10 sharp, nonshifting bands for MW determination on gels and blots
- Natural protein standards are available in high, low, and broad ranges to monitor transfer efficiency and for MW estimation on gels and blots
- IEF standards allow reproducible, dependable pI calibration in native polyacrylamide and agarose IEF gels
- 2-D SDS-PAGE standards provide calibrated references for the pI and MW of proteins in 2-D SDS-PAGE applications

For more information on our wide selection of standards, visit us on the Web at **www.bio-rad.com/ad/proteinstandards/**

Precision Plus Protein™ Standards Family



SPECIAL SECTION

Modeling the Mind

SCIENCE CAREERS

www.sciencecareers.org CAREER RESOURCES FOR SCIENTISTS

GLOBAL: Special Feature—Careers in Computational Neuroscience
A. Kotok
Career and funding opportunities are growing for researchers who combine neuroscience with informatics and engineering.

EUROPE: Leading the Blue Brain Project
E. Pain
Postdoc Felix Schürmann leads the Blue Brain Project, a joint effort of IBM and the Brain Mind Institute to model the mammalian brain.

GLOBAL: Financing Your Research in Computational Neuroscience
A. Kotok
Read about the leading American sources of funding for research and training in computational neuroscience.

SCIENCE'S STKE

www.stke.org SIGNAL TRANSDUCTION KNOWLEDGE ENVIRONMENT

EDITORIAL GUIDE: Focus Issue—Exciting Times for Signaling in the Brain
E. M. Adler
Glutamate receptors are central to brain function and pathology.

PERSPECTIVE: D-Serine Regulation of NMDA Receptor Activity
H. Wolosker
Does D-serine released from neurons and glia play distinct roles in regulating NMDA receptor activity?

REVIEW: A Unified Model of the Presynaptic and Postsynaptic Changes During LTP at CA1 Synapses
J. Lisman and S. Raghavachari
A structural model for long-term potentiation accounts for seemingly contradictory results.

REVIEW: Molecular Signaling Mechanisms Underlying Epileptogenesis
J. O. McNamara, Y. Z. Huang, A. S. Leonard
Calcium in dendritic spines may play a key role in epileptogenesis.

Geologists debate which gases caused trances.

SCIENCENOW

www.sciencenow.org DAILY NEWS COVERAGE

The Prophet of Gases
Geologists are debating what kinds of fumes might have inspired an ancient Greek story about a trance-inducing temple.

Why Did the Lion Lose His Mane?
New report may solve mystery of 'maneless' lions in Kenya.

Is Radiation Contagious?
X-rayed fish may pass ill effects onto their unexposed companions.

Scandal sparks ethics conference.

SCIENCE CAREERS

www.sciencecareers.org CAREER RESOURCES FOR SCIENTISTS

US: Who We Are As Scientists
B. Benderly
Spurred by the stem-cell scandal, a group of young Korean scientists organized an international conference on ethical issues.

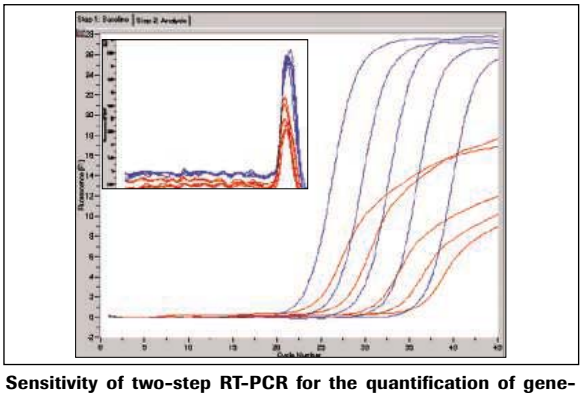
CREDIT (SCIENCENOW IMAGE): BETSY MASON

Separate individual or institutional subscriptions to these products may be required for full-text access.

www.roche-applied-science.com

Transcriptor First Strand cDNA Synthesis Kit

Bring Power and Sensitivity *to Two-Step qRT-PCR*



Sensitivity of two-step RT-PCR for the quantification of gene-expression levels on the LightCycler® 2.0 Instrument. Either the Transcriptor First Strand cDNA Synthesis Kit (blue curves) or another supplier's RNase H⁻ M-MuLV Reverse Transcriptase (red curves) was used to perform reverse transcription on 100 ng to 10 pg of total RNA from K-562 cells. LightCycler® FastStart DNA MasterPLUS SYBR Green I† was then used with primers for G6PDH to quantify gene-expression levels on the LightCycler® 2.0 Instrument.

Choose the new **Transcriptor First Strand cDNA Synthesis Kit** for sensitive results from standard and difficult templates in two-step RT-PCR on blockcyclers and real-time PCR instruments (*e.g.*, the LightCycler® Instruments or ABI PRISM Instruments).

- **Increase sensitivity in quantitative RT-PCR**
  Improve gene-expression studies by increasing sensitivity (see figure) and simultaneously reverse transcribing rare and abundant RNA (over a 10^8-fold range of RNA)–without distorting gene-expression levels.
- **Power through challenging RNA secondary structures**
  Reverse transcribe at 55°C to obtain RT-PCR products from templates that M-MuLV reverse transcriptases can't handle.
- **Produce large quantities of full-length cDNA in qualitative two-step RT-PCR**
  Achieve high yields of cDNAs up to 12 kb using the kit's anchored-oligo$(dT)_{18}$ primer, which prevents mispriming within the poly(A) tail.

To maximize power and sensitivity in two-step RT-PCR, insist on the new Transcriptor First Strand cDNA Synthesis Kit.

For more information, visit *www.roche-applied-science.com/pcr* or contact your local Roche representative.

Transcriptor First Strand cDNA Synthesis Kit		
Cat. No.	04 379 012 001	1 kit for 50 reactions

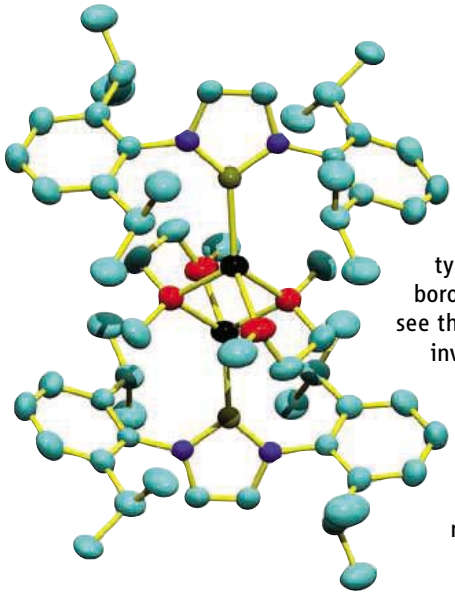
Transcriptor Reverse Transcriptase		
Cat. No.	03 531 317 001	25 reactions
	03 531 295 001	50 reactions
	03 531 287 001	200 reactions

† Purchase of this product is accompanied by a limited license to use it in the PCR process, including homogeneous PCR methods described in U.S. Patents Nos. 5,994,056, 6,171,785, 6,569,627 and corresponding patents outside the United States, for life science research in conjunction with a thermal cycler whose use in the automated performance of the PCR process is covered by the up-front license fee, *i.e.*, an authorized thermal cycler. No real-time apparatus or system patent rights or any other patent rights owned by Applera Corporation are conveyed expressly, by implication or by estoppel. No rights for any other application, including any *in vitro* diagnostic application, are conveyed expressly, by implication or by estoppel under U.S. Patents Nos. 6,174,670 and 6,245,514 and corresponding patent claims outside the United States, or any other patents owned by Roche Molecular Systems, Inc. and F. Hoffmann-La Roche Ltd, claiming real-time amplification and detection methods. The product is covered in-part by US 5,871,908, co-exclusively licensed from Evotec OAI AG.
The technology used for the LightCycler® System is licensed from Idaho Technology Inc., Salt Lake City, UT, USA.

Roche Diagnostics

Roche Diagnostics GmbH
Roche Applied Science
68298 Mannheim
Germany

EDITED BY STELLA HURTLEY AND PHIL SZUROMI



Boron Goes Negative

The chemistry of boron is generally characterized by electron deficiency. Neutral boron compounds tend to be strong Lewis acids, and in borate anions, the B center is typically electropositive, which is reflected in the utility of borohydride salts as hydride (H^-) donors. **Segawa *et al.*** (p. 113; see the Perspective by **Marder**) have prepared a molecule that inverts this reactivity pattern. By reductive cleavage of a B–Br bond in a cyclic precursor, they isolate a boryl lithium compound in which the negatively charged B center acts as a base and nucleophile. The compound is stabilized by electron-donating nitrogens that flank the B atom, in analogy with the isoelectronic *N*-heterocyclic carbenes that have recently been prepared as ligands.

Relativity Tests

The double-pulsar system PSR J0737-3039A/B, which comprises two radio pulsars that orbit one another quickly and with high acceleration, is the best system that has been identified for tests of general relativity. **Kramer *et al.*** (p. 97, published online 14 September; see the 15 September news story by **Cho**) report precision timing observations for a 3-year period. With mass measurements possible, four independent tests confirm the validity of general relativity at the 0.05% level in the strong-field regime.

From Cradle to Museum

For most major groups of organisms, diversity decreases from the tropics to the poles, which may either reflect greater rates of speciation or of species persistence in the tropics. A large-scale analysis of the fossil record of marine bivalves by **Jablonski *et al.*** (p. 102; see the Perspective by **Marshall**) shows that the present-day latitudinal gradient in biodiversity reflects both higher originations in the tropics and poleward expansions of distributional limits of taxa over time. Thus, the tropics are both a cradle and a museum of biodiversity.

Unraveling Protein Analysis

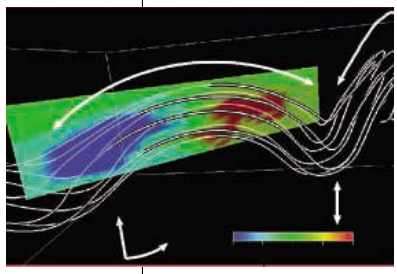
Mass spectrometry is a rapid method for proteomic analysis and for identifying posttranslational modifications. Identification is less ambiguous for "top-down" approaches, where the entire protein is introduced into the gas phase (as opposed to bottom-up approaches that analyze proteolytic fragments). However, fragmentation of the protein becomes more difficult for larger proteins, and top-down approaches are usually limited to proteins with masses below 50 kilodaltons (kD). **Han *et al.*** (p. 109; see the Perspective by **Chait**) extend this approach to proteins with masses in excess of 200 kD by adding small molecules to the electrospray solution that inhibits folding and by activating the ions through heating and collisions induced by voltage acceleration. This process creates hundreds of interresidue cleavages at the C- and N-terminal ends of the chain that allow for identification of larger proteins and provides sufficient mass resolution to assign features such as disulfide linkages.

Monsoon Forecasting

The agricultural output of India depends heavily on the strength of the summer monsoon. Most seasonal forecasts scale the strength of the monsoon to the intensity of the El Niño–Southern Oscillation, a strategy that works well enough predicting heavy rains when equatorial Pacific sea surface temperatures are cold but not nearly as well in predicting drought when temperatures are warm. **Krishna Kumar *et al.*** (p. 115, published online 7 September) used Indian rainfall records extending back more than 130 years and an atmospheric general-circulation model to show that drought occurrence in central India depends on whether the warmest sea surface temperatures occur in the central or the eastern parts of the equatorial Pacific Ocean. Incorporating this result into forecasts of the monsoon could have a significant effect on economic planning and risk mitigation.

Magnetic Loops

The center of the Milky Way Galaxy not only harbors a black hole, but within the inner hundreds of parsecs lie many unusual features, including oddly shaped gas filaments and regions of hot molecular gas with very violent motions. **Fukui *et al.*** (p. 106; see the Perspective by **Morris**) obtained a sequence of CO spectral-line images of the region taken at millimeter wavelengths that reveal vast loops of fast-moving molecular gas. They suggest the loops are expelled by magnetic buoyancy effects similar to those on the Sun's surface. Modeling shows that this magnetic picture can explain the high velocity dispersions of the hot-gas regions.



Gradual Versus Punctuational Evolution

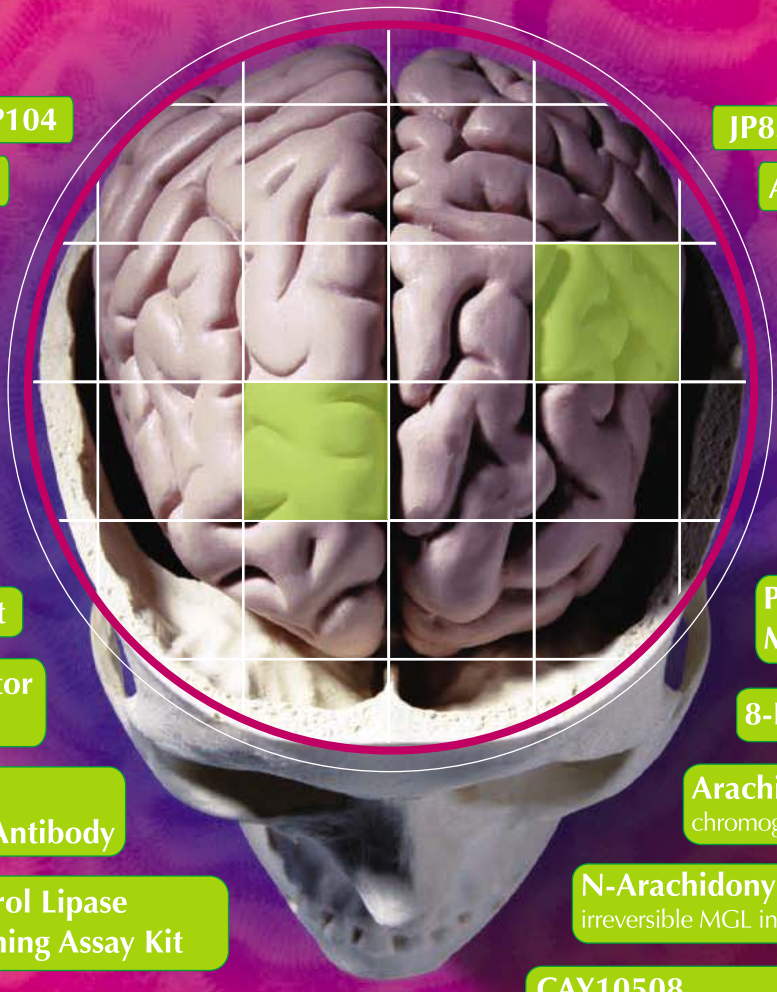
Punctuational evolution has been a contentious idea, but recently, it has become possible to detect signals of punctuational and gradual evolution on molecular phylogenetic trees. **Pagel *et al.*** (p. 119) show that bursts of evolution associated with speciation across a wide range of organisms account for approximately 20% of the nucleotide substitu-

Continued on page 15

CREDITS (TOP TO BOTTOM): SEGAWA *ET AL.*; FUKAI *ET AL.*

ENDOCANNABINOIDS

& RELATED NEUROCHEMICALS



NEW **JP104**

URB597

cAMP EIA Kit
cGMP EIA Kit
CGRP EIA Kit

NEW **Human GFAP EIA Kit**

NAPE-PLD Polyclonal Antibody

Substance P EIA Kit

CB_1 & CB_2 Receptor Antibodies

Monoacylglycerol Lipase Polyclonal Antibody

NEW **Monoacylglycerol Lipase Inhibitor Screening Assay Kit**

S-Nitrosylated Protein Detection Kit

JP83 NEW

AM404

AEA & 2-AG

Gabapentin

CAY10429
abnormal cannabidiol

Capsazepine
$TRPV_1$ antagonist

Prion Protein Monoclonal Antibodies

8-Isoprostane EIA Kit

Arachidonoyl-1-Thio-Glycerol
chromogenic MGL substrate

N-Arachidonyl Maleimide NEW
irreversible MGL inhibitor

CAY10508 NEW
potent CB_1 receptor inverse agonist

And Many More...
www.caymanchem.com/neuroscience

Cayman CHEMICAL

800.364.9897 • www.caymanchem.com

Continued from page 13

tions in published gene-sequence alignments, the remainder being attributable to more gradual forces of evolution. These "punctuational" effects are more frequent in plants and fungi, compared with animals, presumably because of their higher rates of polyploidy and hybridization.

Tale of Two Two-Timing Proteins

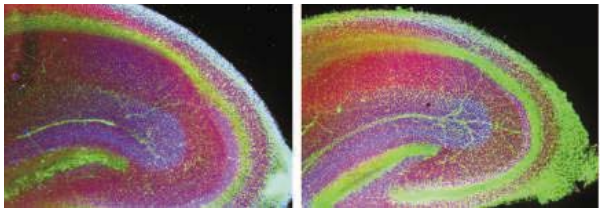
Cells put many proteins to more than one use, including the transcription factor TFII-I. **Caraveo *et al.*** (p. 122; see the Perspective by **Park and Dolmetsch**) found that TFII-I may inhibit calcium entry into cells by interacting with another protein that itself has dual roles in regulating cellular calcium concentrations—phospholipase C–γ (PLC-γ). PLC-γ promotes the generation of inositol-1,4,5-trisphosphate, a second messenger that causes calcium release from intracellular stores, and also interacts with the membrane calcium channel TRPC3 and promotes insertion of the channel into the plasma membrane. The interaction of TFII-I with PLC-γ appears to depend on TFII-I's phosphorylation, which may keep PLC-γ from interacting with the TRPC3 channel and thereby limit calcium entry through the plasma membrane.

Immune Gene Therapy for Cancer

Cancer immunotherapy is based on the premise that the sometimes reluctant immune system can be cajoled into efficiently destroying tumors, either through vaccination or the adoptive transfer of cancer-killing cells. **Morgan *et al.*** (p. 126, published online 31 August; see the Perspective by **Offringa**) genetically modified T cells to express a T cell receptor (TCR) with strong specificity for a selected melanoma tumor antigen. Peripheral blood lymphocytes isolated from patients with advanced metastatic melanoma were transduced with a retroviral vector containing genes of the two chains of the TCR. After re-infusion, the transgenic cells were maintained and, encouragingly, 2 of the original 17 patients carrying the highest numbers of cells also responded with a noticeable regression of their established tumors.

Channeling Local Translation

Local, activity-dependent changes in excitability are thought to play a role in various aspects of neuromodulation. Translation of dendritically localized messenger RNAs is one way for a neuron to modify its excitability and signal processing machinery at or near active synapses. **Raab-Graham *et al.*** (p. 144) used photoconvertible fluorophores to show that potassium channels are locally translated in dendrites. This local translation is regulated by *N*-methyl-D-aspartate receptor activation via the mammalian target of rapamycin/phosphatidylinositol 3-kinase signaling pathway.



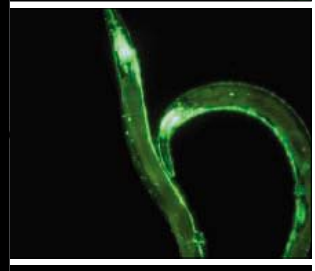
CREDITS (TOP TO BOTTOM): RAAB-GRAHAM *ET AL.*

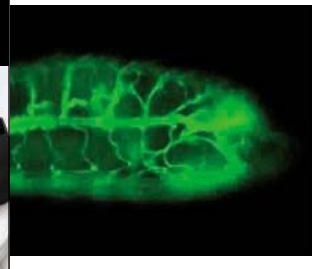
Chronic Wasting Disease Transmission

Chronic wasting disease (CWD) is a fatal prion disease found in deer and elk. Its transmission between animals seems to be much easier than that of so-called mad cow disease between cattle. **Mathiason *et al.*** (p. 133) demonstrate the presence of infectious prions capable of transmitting CWD in body fluids, including the saliva and the blood, of CWD-infected cervids. The results emphasize the need for caution regarding contact with body fluids of infected animals.

Anxious Mice and Men

The genes that contribute to depression and anxiety disorders are still unknown, but the recently discovered single-nucleotide polymorphism (Val66Met) in the brain-derived neurotrophic factor (BDNF) gene may be related to mood and anxiety disorders common in human populations. **Chen *et al.*** (p. 140) report that, in transgenic mice expressing the variant $BDNF_{Met}$ version, there are alterations in brain anatomy and memory as has been described in humans. This allelic variant also reproduces the phenotypic hallmarks of anxiety in humans, but these mutant mice did not respond to a common, widely used antidepressant.





Competence in Fluorescence

Just let the world's best fluorescence stereomicroscopes work for you with precision, speed and brilliance. You determine the sequence of your fluorescence experiments. The Leica Macroscope, MZ10 F, MZ16 F or MZ16 FA offer the top performance of your needs: 10:1 up to 16:1 zoom, maximum magnification of 920x, resolution of up to 1500 lp/mm, finest visible structure width of up to 0.3 µm.

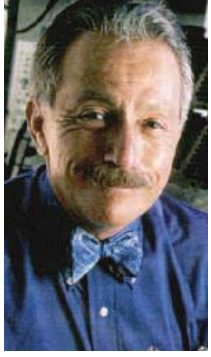
Automated performance guarantees higher productivity and better results in industry and life science research environments. Leica fluorescence instruments ultimately safeguards the future of your business.

The Leica Macrofluo, MZ10 F, MZ16 F and MZ16FA can be individually combinded with the Leica LAS software, Leica digital cameras and ergonomic accessories to a complete solution for perfect documentation and analysis.

www.leica-microsystems.com/**stereomicroscopes**

Leica

MICROSYSTEMS

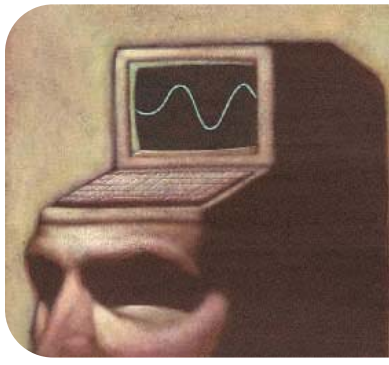


Floyd Bloom is a professor emeritus at The Scripps Research Institute, La Jolla, CA, and served as *Science*'s Editor-in-Chief from 1995 to 2000. He works in the field of neuropharmacology. E-mail: fbloom@scripps.edu

Prying Open the Black Box

IF THE ABSTRACTS FOR THIS YEAR'S ANNUAL MEETING OF THE SOCIETY FOR NEUROSCIENCE (later this month in Atlanta, Georgia) are any indication, collaboration between experimentalists and theoreticians is thriving. This is good news for neuroscience, given that not so long ago, neuroscientists who did experiments the old-fashioned way—recording single-cell activity in living, often behaving experimental animals—had little tolerance for theoreticians of the brain. Both groups hoped to understand how the brain works, but the theoreticians apparently wanted to achieve that goal while ignoring the complex and dynamic ways in which neurons communicate. The pervasive view among the experimentalists was that for the theoreticians, the brain may as well be a black box. Well, that sentiment is no longer pervasive. Rather, computational neuroscience (see the special section in this issue) has made great strides in the past decade, and the field is poised to resolve a spectrum of stubborn mysteries of the brain.

The sources of the old experimental/theoretical divide are understandable. Most neuroscientists trained in the 1960s, 1970s, and 1980s were comfortable with mathematical representations of basic neuronal phenomena, such as the ionic basis for resting membrane potential. They were awed by the elegant Hodgkin-Huxley equations that explained and predicted the ionic basis for the action potential (based, of course, on an ideal experimental model, the squid giant axon). Experimental neuroscience even embraced the quantal theory of neurotransmitter release by neurons. However, when the theorists began turning their attention to how the cerebellum or hippocampus works, these theories that avoided the complexities of neuronal circuitry and synaptic interactions were suddenly regarded as too speculative and not testable.



During the past decade, there has been a robust explosion of methods to characterize the functional and structural details of neurons and their interconnected cellular networks. We can finally realistically simplify the essential features of neuronal ensembles and test theories about what specific neuronal circuits may do. In other words, we can ask why specific cortical pyramidal neurons and interneurons underlie processes such as "edge detection." A wealth of quantitative data on signaling mechanisms, ion-channel behavior, neuron populations, and morphological features has made possible the development of neuronal and synaptic models that are rigorously constrained by the biochemical and structural properties of specific types of neurons. Such models have wedged open the black box, allowing experimentalists and theoreticians to collaborate. And, as reflected by the Society for Neuroscience meeting agenda, the targets of computational neuroscience are wide-ranging, speaking not only to age-old questions about the possible information encoded in intervals of electrical activity in neurons but also to decision-making and risk/reward assessment and neurological (mainly epilepsy) and psychiatric (schizophrenia and depression) diseases. Computational neuroscience permits us to ask what structural principles explain the selective and redundant wiring diagrams of single-sense pathways and those that integrate multiple senses.

Computational neuroscience is also attempting to incorporate the burgeoning field of neuroinformatics and the growing body of electronic databases into its evolution. This is where some of the next great challenges lie. We need to develop and implement uniform, standardized modes of data presentation in neuroscience, so that data from individual research papers can be readily scanned and integrated into more comprehensive databases. This will be fundamental to developing more accurate and useful models of brain function. For many years, the U.S. National Institutes of Health funded the Human Brain Project to nurture this field, and comparable efforts were established globally. However, the funds for ongoing maintenance do not exist, and past investments are endangered.* If adequate funding over a prolonged period of time is not secured, new principles of education, psychology, and social science that are based on neuroscience are in jeopardy.

– Floyd Bloom

10.1126.science.1135216

*M. S. Gazzaniga *et al.*, *Science* **311**, 176 (2006).

CREDIT: GETTY IMAGES

"I think the dosage needs adjusting. I'm not nearly as happy as the people in the ads."

It's time for genetics.

What if you could use DNA to identify patients who respond well to your drug? You might use that knowledge to reach more patients and expand your market, or to get a drug to market faster. Either way – patients win. Perlegen is helping drug companies do just that – today.

Working with you, we can comprehensively analyze the DNA from thousands of patients taking your drug. Out of the millions of genetic variations between patients, we may be able to help you identify the ones that are associated with strong efficacy, poor efficacy, or side effects. Perlegen's exceptional knowledge of the genome and experienced team of analysts could help you get clinically relevant answers, not just data, in a matter of months.

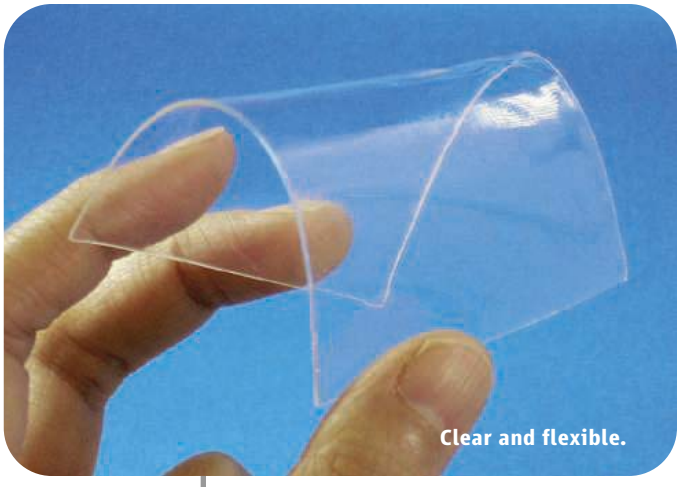
We partner with the top pharmaceutical companies around the world. We also license late-stage drugs. If you have a drug that can benefit from our approach, please contact us.

Patients are waiting.

genetics@perlegen.com
Mountain View, California • 650-625-4500
Tokyo, Japan • +81 (0)3 3444-6080
www.perlegen.com

Targeting today's drugs. Discovering tomorrow's.™

PERLEGEN SCIENCES®



MATERIALS SCIENCE

Stretchy Clay Composites

Clays are considered useful reinforcing materials on account of the individual platelets exposed through exfoliation; these platelets offer a large surface area for chemical bonding. However, the synthesis of a composite material by the addition of large amounts of clay to a polymer is problematic because of dispersion or aggregation effects that lead to poor mechanical or optical properties. Haraguchi *et al.* used gel formation in an aqueous medium to create a composite of hydrophobic poly(2-methoxyethyl acrylate) and hydrophilic hectorite clay. During the polymerization, the clay platelets were excluded from the polymer particles and instead formed a shell around them. Once dried, the clay shells comprised a three-dimensional network, which the authors structurally characterized using transmission electron microscopy.

A surprising feature of the composites was the ability to undergo huge elongations when subjected to stress. After an initial irreversible necking deformation, subsequent applied large strains were shown to be reversible, with good shape recovery observed on release. The composites were also transparent, with greater than 90% light transmission independent of clay concentration (up to 30 weight %). Unlike many clay composites, these materials did not reswell on exposure to water, nor did they dissolve in organic solvents that could solubilize the pure polymer. — MSL

Adv. Mater. **18,** 2250 (2006).

CHEMISTRY

Salt Solution

Ionic liquids (ILs), or more specifically cation-anion pairs that form a stable fluid near room temperature, are playing an increasingly practical role as chemical reaction solvents, electrolytes, and heat-transfer media on scales that range from the laboratory bench to industrial manufacturing processes. Their advantages include miniscule vapor pressure, high polarity, and relative inertness. However, efforts to fine-tune IL properties by structural modification are hindered by a limited understanding of why specific cation-anion combinations melt at such low temperature.

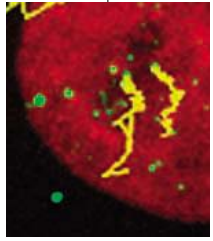
Krossing *et al.* have developed a simple predictive framework for calculating the melting point of a given IL with knowledge of the dielectric constant, or conversely estimating dielectric properties from the melting point. Their method involves computing the free energy of fusion using a thermodynamic cycle that adds the lattice energy (required to move ions from the lattice to the gas phase) to the compensating stabilization energy arising from naked ion solvation in a dielectric medium. Enthalpic and entropic parameters are calculated using a combination of quantum chemical methods and volume-based-thermodynamics approximations. The modeling scheme proved highly effective in reproducing experimental data for 14 ionic liquids composed of the most commonly used cations (substituted imidazolium, pyrrolidinium, pyridinium, and ammonium) and anions [BF_4^-, PF_6^-, $CF_3SO_3^-$, and $(CF_3SO_2)_2N^-$]. — JSY

J. Am. Chem. Soc. **128**, 10.1021/ja0619612 (2006).

VIROLOGY

Caught in the Act

Real-time intracellular imaging allows detailed visualization of viral entry mechanisms. Arhel *et al*. have attached a tetracysteine tag to the integrase protein of human immunodeficiency virus–1 (HIV-1). The tagged viruses retain infectivity and can be labeled with a fluorophore, allowing real-time tracking of individual viral DNA–containing complexes in the cytoplasm and nucleus of human cells. Before entering the nucleus, the viral particles exhibited actin- and microtubule-based movement from the periphery toward the nucleus. Their mobility was restrained as they docked with and crossed the nuclear envelope, and the particles were then able to move diffusively once inside the nucleus itself. This type of technology will be important for identifying the itineraries of viruses during infection and for testing potential interventions that would interfere with the establishment of productive infections. — SMH



Viral particles (green) and their trajectories (yellow) toward the nucleus (red).

Nat. Methods **3**, 817 (2006).

OCEAN SCIENCE

Shallow Chills

Observations show that the world oceans as a whole have been warming for the past 50 years. This result is an important confirmation of global warming inferences based on surface atmospheric temperature measurements, as the oceans have more than a thousand times the heat capacity of the atmosphere. The rise in ocean heat content has not been spatially or temporally uniform, however, and because most models do not reproduce such unforced variations, their origin remains an open question.

Lyman *et al.* have taken advantage of the rapidly expanding network of Argo autonomous profiling floats to present a global temperature data set for the upper 750 m of the world oceans. The study reveals a large cooling since 2003. These data also have implications beyond the pattern and extent of cooling. For instance,

Continued on page 21

CREDITS (TOP TO BOTTOM): HARAGUCHI *ET AL.*, *ADV. MATER.* **18**, 2250 (2006); ARHEL *ET AL.*, *NAT. METHODS* **3**, 817 (2006)

Journals IMPACTING Science

Current Drug Metabolism

I.F. 5.42

"Current Drug Metabolism represents an important review journal of great value to pharmaceutical scientists."

(Donald Everett
Bristol-Myers Squibb, USA)

Volume 7, 8 Issues, 2006
Personal Subscription: $ 280.00

Current Drug Targets

I.F. 4.40

"In view of the growing volume of literature, the role of high quality review journals has become increasingly important. Current Drug Targets is an important journal in the field of medicinal chemistry and drug design, which is strongly recommended to the scientific community."

(Jean-Marie Lehn
Nobel Laureate)

Volume 7, 12 Issues, 2006
Personal Subscription: $ 420.00

Current Protein & Peptide Science

I.F. 4.15

"This journal presents the latest developments in the area of protein chemistry. This is strongly recommended."

(Robert Huber
Nobel Laureate)

Volume 7, 6 Issues, 2006
Personal Subscription: $ 210.00

Current HIV Research

I.F. 3.00

publishes comprehensive reviews on all areas of HIV research. Periodically, the journal invites Guest Editors to devote an issue to a topical area of HIV research, with the aim to increase our understanding of the virus and its complex interaction with the host cell.

Volume 4, 4 Issues, 2006
Personal Subscription: $ 140.00

Combinatorial Chemistry & High Throughput Screening

I.F. 2.52

"Combinatorial Chemistry & High Throughput Screening is a pivotal journal in the field of drug discovery."

(Norman R. Farnsworth
University of Illinois at Chicago, USA)

Volume 9, 10 Issues, 2006
Personal Subscription: $ 310.00

Current Organic Chemistry

I.F. 3.10

"Current Organic Chemistry is an important review journal, which should prove to be of wide interest to organic chemists and provide them with a convenient means of keeping up with the current flood of advances in the field."

(H.C. Brown
Nobel Laureate)

Volume 10, 18 Issues, 2006
Personal Subscription: $ 470.00

Current Pharmaceutical Design

I.F. 4.83

"This journal is a necessity for scientists working in the multi-disciplinary fields encompassing the areas of drug design and discovery."

(Annette M. Doherty,
Pfizer Global R&D, France)

Volume 12, 36 Issues, 2006
Personal Subscription: $ 1080.00

Current Topics in Medicinal Chemistry

I.F. 4.40

"Current Topics in Medicinal Chemistry provides medicinal chemists and scientists in allied disciplines an invaluable resource for thematic coverage of keen new developments in their field of study."

(Sir James Black
Nobel Laureate)

Volume 6, 18 Issues, 2006
Personal Subscription: $ 410.00

Current Molecular Medicine

I.F. 4.94

"A critically important journal that fills an important niche."

(David Weiner
Pennsylvania School of Medicine, USA)

Volume 6, 8 Issues, 2006
Personal Subscription: $ 280.00

Current Medicinal Chemistry

I.F. 4.90

"Current Medicinal Chemistry has established itself as an important review journal in the field of medicinal chemistry. The journal is highly recommended to all scientists active in the field of medicinal chemistry."

(Richard R. Ernst
Nobel Laureate)

Volume 13, 30 Issues, 2006
Personal Subscription: $ 740.00

Get your FREE copy online now!

www.bentham.org

For Information or to Subscribe:

subscriptions@bentham.org

Tel: +971 65571132; Fax: +971 66571134

BENTHAM SCIENCE PUBLISHERS LTD.

Continued from page 19

because it is unlikely that so much heat was transferred so quickly to the deeper ocean, the measurements indicate that a whole-ocean cooling has occurred, a phenomenon expected to induce a decrease in sea level due to thermal contraction of the water. Sea level rise has not slowed during the time period, however, suggesting that other factors such as increased rates of glacial melting are more than adequate to compensate for the thermal effect on volume. — HJS

Geophys. Res. Lett. **33**, L18604 (2006).

BIOCHEMISTRY

How to Handle Methane

The biological oxidation of methane to carbon dioxide proceeds sequentially—through methanol, formaldehyde, and formic acid. Carrying out these reactions under mild conditions demands exquisite control, which generally translates into precisely structured metal centers and diffusion-restricted intermediates. Myronova *et al.* have isolated an enzyme complex containing the membrane-bound (or particulate) form of methane monooxygenase (pMMO) and methanol dehydrogenase (MDH), and they map its structure using cryoelectron microscopy. They are able to fit three copies of the previously solved crystal structure of pMMO, an enzyme with mononuclear and binuclear copper centers, into the body of a 500-kD assembly; likewise, three copies of the crystal structure of MDH can be fitted into the cap, which is connected to the body via three arms. This supramolecular organization may facilitate the controlled transfer of electrons, which appears to be a common theme among membrane-bound enzymes catalyzing redox chemistry. — GJC

A cutaway view of the pMMO-MDH complex.

CREDIT: MYRONOVA *ET AL.*, *BIOCHEMISTRY* **45**, 10.1021/BI061294P (2006)

Biochemistry **45**, 10.1021/bi061294p (2006).

GENETICS

Silencing in Triplicate

The movement and activity of transposons, also known as jumping genes for their ability to replicate and move throughout the genome, have long fascinated biologists. In some cases, transposon activity is reduced, and more recently it has been demonstrated that this reduction is often due to epigenetic silencing and paramutation: the epigenetic change in expression of one allele effected by another.

Woodhouse *et al.* propose that the establishment, maintenance, and inheritance silencing of the *MuDR* transposon in maize are due to multiple genes. These genes include *Mu killer*, a transcribed template for RNA interference silencing that initiates silencing; a homolog of the *Arabidopsis RDR2* gene, named *ZmRDR2/Mediator of paramutation 1*, which is required to initiate silencing in the cases where a double-stranded RNA hairpin is lacking and perpetuates silencing through RNA-directed DNA methylation; and two maize homologs of nucleosome assembly protein 1, which maintain heritable silencing, most likely by modification of the chromatin structure. The redundant mechanisms involved in regulating transposon activity demonstrate the importance of controlling the replication of parasitic DNA elements within the host genome, which has been suggested as the raison d'être for epigenetic silencing. — LMZ

PLoS Biol. **4**, e339 (2006).

Science STKE AAAS

www.stke.org

<< Inflammatory Pores

As part of the process of infection, pathogenic microbes secrete protein toxins, such as aerolysin, that form pores in the target cell's membrane. Gurcel *et al.* show that exposure of mammalian cells to aerolysin increased the activity of the transcription factor SREBP-2 (sterol response element–binding protein-2), as assessed by a selective increase in gene expression and an increase in total cellular cholesterol as a consequence of both increased biosynthesis and uptake (the low-density lipoprotein receptor is encoded by a SREBP-responsive gene). In addition, exposure of cells to a K^+-selective ionophore triggered SREBP-2 activation, and a decrease in intracellular K^+ (caused by leakage through the pores) is known to activate the protease caspase 1 via the assembly of inflammasomes containing IPAF, an intracellular pattern recognition receptor. SREBP-2 was not cleaved directly by caspase 1; instead, caspase 1 activated the SREBP-activating pathway that involves SCAP (an escort protein) and S1P and S2P (the two enzymes responsible for SREBP proteolysis and release). Using various pharmacological and RNA interference approaches, the authors showed that activation of the inflammasome, caspase 1, and the SREBPs was required for cell survival after exposure to aerolysin. — NRG

Cell **126**, 1135 (2006).

you've got **better** things to do...

better consistency

better purification

better results

Introducing
Personal Automation™

Meet Maxwell

Maxwell™ 16 gives you consistent purification results — processing up to 16 samples in 30 minutes. It's Personal Automation™, right at your lab bench. DNA, RNA or protein purification, your choice. Finally reagents, instrumentation, service and support from one reliable source. You'd better visit: **www.MeetMaxwell.com**

Promega

www.sciencemag.org

Science

1200 New York Avenue, NW
Washington, DC 20005
Editorial: 202-326-6550, FAX 202-289-7562
News: 202-326-6500, FAX 202-371-9227

Bateman House, 82-88 Hills Road
Cambridge, UK CB2 1LQ
+44 (0) 1223 326500, FAX +44 (0) 1223 326501

Subscription Services For change of address, missing issues, new orders and renewals, and payment questions: 866-434-AAAS (2227) or 202-326-6417, FAX 202-842-1065. Mailing addresses: AAAS, P.O. Box 96178, Washington, DC 20090-6178 or AAAS Member Services, 1200 New York Avenue, NW, Washington, DC 20005

Institutional Site Licenses please call 202-326-6755 for any questions or information

Reprints: Author Inquiries 800-635-7181
Commercial Inquiries 803-359-4578
Corrections 202-326-6501

Permissions 202-326-7074, FAX 202-682-0816

Member Benefits Bookstore: AAAS/BarnesandNoble.com bookstore www.aaas.org/bn; Car purchase discount: Subaru VIP Program 202-326-6417; Credit Card: MBNA 800-847-7378; Car Rentals: Hertz 800-654-2200 CDP#343457, Dollar 800-800-4000 #AA1115; AAAS Travels: Betchart Expeditions 800-252-4910; Life Insurance: Seabury & Smith 800-424-9883; Other Benefits: AAAS Member Services 202-326-6417 or www.aaasmember.org.

science_editors@aaas.org	(for general editorial queries)
science_letters@aaas.org	(for queries about letters)
science_reviews@aaas.org	(for returning manuscript reviews)
science_bookrevs@aaas.org	(for book review queries)

Published by the American Association for the Advancement of Science (AAAS), *Science* serves its readers as a forum for the presentation and discussion of important issues related to the advancement of science, including the presentation of minority or conflicting points of view, rather than by publishing only material on which a consensus has been reached. Accordingly, all articles published in *Science*—including editorials, news and comment, and book reviews—are signed and reflect the individual views of the authors and not official points of view adopted by the AAAS or the institutions with which the authors are affiliated.

AAAS was founded in 1848 and incorporated in 1874. Its mission is to advance science and innovation throughout the world for the benefit of all people. The goals of the association are to: foster communication among scientists, engineers and the public; enhance international cooperation in science and its applications; promote the responsible conduct and use of science and technology; foster education in science and technology for everyone; enhance the science and technology workforce and infrastructure; increase public understanding and appreciation of science and technology; and strengthen support for the science and technology enterprise.

Information for Contributors

See pages 102 and 103 of the 6 January 2006 issue or access www.sciencemag.org/feature/contribinfo/home.shtml

EDITOR-IN-CHIEF **Donald Kennedy**
EXECUTIVE EDITOR **Monica M. Bradford**
DEPUTY EDITORS **R. Brooks Hanson, Barbara R. Jasny, Katrina L. Kelner**
NEWS EDITOR **Colin Norman**

Editorial supervisory senior editor Phillip D. Szuromi; **senior editor/perspectives** Lisa D. Chong; **senior editors** Gilbert J. Chin, Pamela J. Hines, Paula A. Kiberstis (Boston), Marc S. Lavine (Toronto), Beverly A. Purnell, L. Bryan Ray, Guy Riddihough, H. Jesse Smith, Valda Vinson, David Voss; **associate editors** Jake S. Yeston, Laura M. Zahn; **online editor** Stewart Wills; **associate online editor** Tara S. Marathe; **book review editor** Sherman J. Suter; **associate letters editor** Etta Kavanagh; **information specialist** Janet Kegg; **editorial manager** Cara Tate; **senior copy editors** Jeffrey E. Cook, Cynthia Howe, Harry Jach, Barbara P. Ordway, Jennifer Sills, Trista Wagoner; **copy editor** Peter Mooreside; **editorial coordinators** Carolyn Kyle, Beverly Shields; **publication assistants** Ramatoulaye Diop, Chris Filiatreau, Joi S. Granger, Jeffrey Hearn, Lisa Johnson, Scott Miller, Jerry Richardson, Brian White, Anita Wynn; **editorial assistants** Lauren Kmec, Patricia M. Moore; **executive assistant** Sylvia S. Kihara; **administrative support** Maryrose Police

News senior correspondent Jean Marx; **deputy news editors** Robert Coontz, Jeffrey Mervis, Leslie Roberts, John Travis; **contributing editors** Elizabeth Culotta, Polly Shulman; **news writers** Yudhijit Bhattacharjee, Adrian Cho, Jennifer Couzin, David Grimm, Constance Holden, Jocelyn Kaiser, Richard A. Kerr, Eli Kintisch, Andrew Lawler (New England), Greg Miller, Elizabeth Pennisi, Robert F. Service (Pacific NW), Erik Stokstad; Rhituparna Chatterjee (intern); **contributing correspondents** Barry A. Cipra, Jon Cohen (San Diego, CA), Daniel Ferber, Ann Gibbons, Robert Irion, Mitch Leslie (NetWatch), Charles C. Mann, Evelyn Strauss, Gary Taubes, Ingrid Wickelgren; **copy editors** Linda B. Felaco, Rachel Curran, Sean Richardson; **administrative support** Scherraine Mack, Fannie Groom **bureaus:** Berkeley, CA: 510-652-0302, FAX 510-652-1867, New England: 207-549-7755, San Diego, CA: 760-942-3252, FAX 760-942-4979, Pacific Northwest: 503-963-1940

Production director James Landry; **senior manager** Wendy K. Shank; **assistant manager** Rebecca Doshi; **senior specialists** Jay Covert, Chris Redwood; **specialist** Steve Forrester **Preflight director** David M. Tompkins; **manager** Marcus Spiegler; **specialist** Jessie Mudjitaba

Art director Joshua Moglia; **associate art director** Kelly Buckheit; **illustrators** Chris Bickel, Katharine Sutliff; **senior art associates** Holly Bishop, Laura Creveling, Preston Huey; **associate** Nayomi Kevitiyagala; **photo editor** Leslie Blizard

***Science* International**

Europe (science@science-int.co.uk) **editorial: international managing editor** Andrew M. Sugden; **senior editor/perspectives** Julia Fahrenkamp-Uppenbrink; **senior editors** Caroline Ash (Geneva: +41 (0) 222 346 3106), Stella M. Hurtley, Ian S. Osborne, Stephen J. Simpson, Peter Stern; **associate editor** Joanne Baker **editorial support** Alice Whaley; Deborah Dennison **administrative support** Janet Clements, Phil Marlow, Jill White; **news: international news editor** Eliot Marshall **deputy news editor** Daniel Clery; **correspondent** Gretchen Vogel (Berlin: +49 (0) 30 2809 3902, FAX +49 (0) 30 2809 8365); **contributing correspondents** Michael Balter (Paris), Martin Enserink (Amsterdam and Paris), John Bohannon (Vienna)

Asia Japan Office: Asca Corporation, Eiko Ishioka, Fusako Tamura, 1-8-13, Hirano-cho, Chuo-ku, Osaka-shi, Osaka, 541-0046 Japan; +81 (0) 6 6202 6272, FAX +81 (0) 6 6202 6271; asca@os.gulf.or.jp; **asia news editor** Richard Stone +66 2 662 5818 (rstone@aaas.org) **japan news bureau** Dennis Normile (contributing correspondent, +81 (0) 3 3391 0630, FAX 81 (0) 3 5936 3531; dnormile@gol.com); **china representative** Hao Xin, + 86 (0) 10 6307 4439 or 6307 3676, FAX +86 (0) 10 6307 4358; haoxin@earthlink.net; **south asia** Pallava Bagla (contributing correspondent +91 (0) 11 2271 2896; pbagla@vsnl.com)

Africa Robert Koenig (contributing correspondent, rob.koenig@gmail.com)

EXECUTIVE PUBLISHER **Alan I. Leshner**
PUBLISHER **Beth Rosner**

Fulfillment & Membership Services (membership@aaas.org) **director** Marlene Zendell; **manager** Waylon Butler; **systems specialist** Andrew Vargo; **customer service supervisor** Pat Butler; **specialists** Laurie Baker, Tamara Alfson, Karena Smith, Vicki Linton, Latoya Casteel; **circulation associate** Christopher Refice; **data entry supervisor** Cynthia Johnson; **specialists** Tomeka Diggs, Tarrika Hill

Business Operations and Administration director Deborah Rivera-Wienhold; **business manager** Randy Yi; **senior business analyst** Lisa Donovan; **business analyst** Jessica Tierney; **financial analyst** Michael LoBue, Farida Yeasmin; **rights and permissions: administrator** Emilie David; **associate** Elizabeth Sandler; **marketing: director** John Meyers; **marketing managers** Darryl Walter, Allison Pritchard; **marketing associates** Julianne Wielga, Mary Ellen Crowley, Catherine Featherston, Alison Chandler, Lauren Lamoureux; **international marketing manager** Wendy Sturley; **marketing/member services executive:** Linda Rusk; **japan sales** Jason Hannaford; **site license sales: director** Tom Ryan; **sales and customer service** Mehan Dossani, Kiki Forsythe, Catherine Holland, Wendy Wise; **electronic media: manager** Lizabeth Harman; **production associates** Amanda K. Skelton, Lisa Stanford, Nichele Johnston; **lead applications developer** Carl Saffell

Advertising director worldwide ad sales Bill Moran

Product (science_advertising@aaas.org); **midwest** Rick Bongiovanni: 330-405-7080, FAX 330-405-7081 • **west coast/w. canada** Teola Young: 650-964-2266 **east coast/e. canada** Christopher Breslin: 443-512-0330, FAX 443-512-0331 • **uk/europe/asia** Tracy Holmes: +44 (0) 1223-326-525, FAX +44 (0) 1223-325-532 **japan** Mashy Yoshikawa: +81 (0) 33235 5961, FAX +81 (0) 33235 5852 **traffic manager** Carol Maddox; **sales coordinator** Deiandra Simms

Commercial Editor Sean Sanders: 202-326-6430

Classified (advertise@sciencecareers.org); **u.s.: recruitment sales manager** Ian King: 202-326-6528, FAX 202-289-6742; **inside sales manager** Daryl Anderson: 202-326-6543; **west coast/midwest** Kristine von Zedlitz: 415-956-2531; **east coast** Jill Downing: 631-580-2445; **canada, meetings and announcements** Allison Millar: 202-326-6572; **sales coordinators** Erika Bryant; Rohan Edmonson, Joyce Scott, Shirley Young; **international: sales manager** Tracy Holmes: +44 (0) 1223 326525, FAX +44 (0) 1223 326532; **sales** Christina Harrison, Svitlana Barnes; **sales assistant** Helen Moroney; **japan:** Jason Hannaford: +81 (0) 52 757 5360, FAX +81 (0) 52 757 5361; **production: manager** Jennifer Rankin; **assistant manager** Deborah Tompkins; **associates** Christine Hall; Amy Hardcastle; **publications assistants** Robert Buck; Mary Lagnaoui

AAAS Board of Directors retiring president, chair Gilbert S. Omenn; **president** John P. Holdren; **president-elect** David Baltimore; **treasurer** David E. Shaw; **chief executive officer** Alan I. Leshner; **board** Rosina M. Bierbaum; John E. Dowling; Lynn W. Enquist; Susan M. Fitzpatrick; Alice Gast; Thomas Pollard; Peter J. Stang; Kathryn D. Sullivan

AAAS
ADVANCING SCIENCE, SERVING SOCIETY

new!

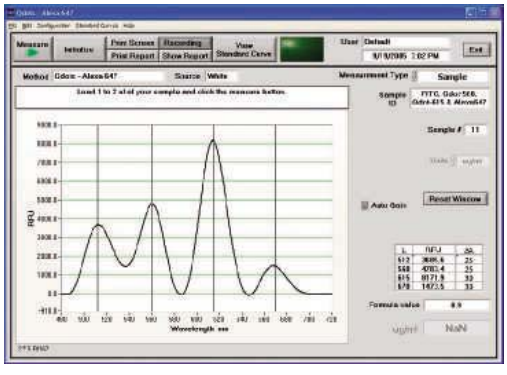
The power of small²

NanoDrop introduces a Fluorospectrometer

1 µl samples ▪ No cuvettes ▪ 10-second measurements ▪ Broad spectral output

Small footprint. Revolutionary technology. The NanoDrop® ND-3300 Fluorospectrometer is a powerful new tool for fluorescence spectrometry. Choose from many pre-defined methods or configure your own.

- **Nucleic acids:** Determine concentration of dsDNA using PicoGreen® assay (2 pg), Quant-iT™ DNA assay or Hoechst 33258 dye; RNA using RiboGreen® dye
- **Proteins:** Determine concentration using Quant-iT™ protein assay
- **More:** FITC (fluorescein), Cy-Alexa Fluor dyes, B-Phycoerythrin, Quinine Sulfate, Sulforhodamine and 4-MU



Measurement is as easy as pipette and read, requiring only 1-2 µl of sample. No cuvettes are necessary — simply wipe the optical surfaces and you're ready for your next sample. A broad excitation range is achieved using UV, blue and white LED sources. The uniquely clean optics of the patented retention system, combined with proprietary white LED signal processing, enables measurements across a wide range of wavelengths without the need for filter changes. The ND-3300 is small, simple and powerful enough for your most challenging and precious samples.

And for the power of small in absorbance measurement, the NanoDrop® ND-1000 UV/Vis Spectrophotometer can detect down to 2ng/µl and up to 3700 ng/µl of dsDNA without dilutions.

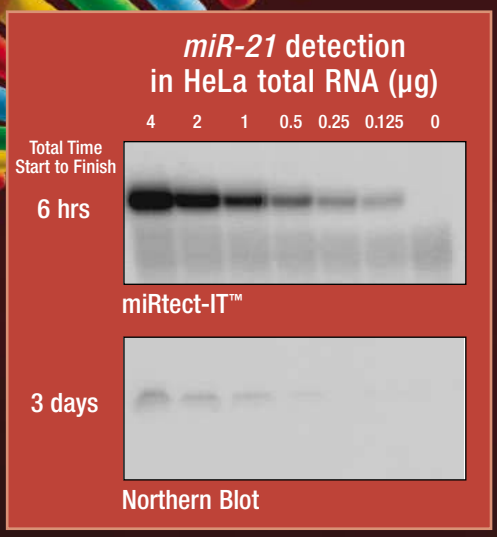
Ready to experience the power of small? Contact us today and try the ND-3300 or ND-1000 in your own lab.

FREE one week evaluation

Call for details **(302)479-7707** **www.nanodrop.com**

NanoDrop

It's a new day in miRNA detection when you can do it in one day.



Announcing USB's new miRtect-IT™ miRNA Labeling and Detection Kit. This highly sensitive, novel method is fast and easy, allowing you to achieve direct labeling and quantitative measurement of miRNAs in less than one day. So don't waste another 3 days using the old Northern Blot method—go to our website today to learn more.

miRtect-IT™ miRNA Labeling and Detection Kit

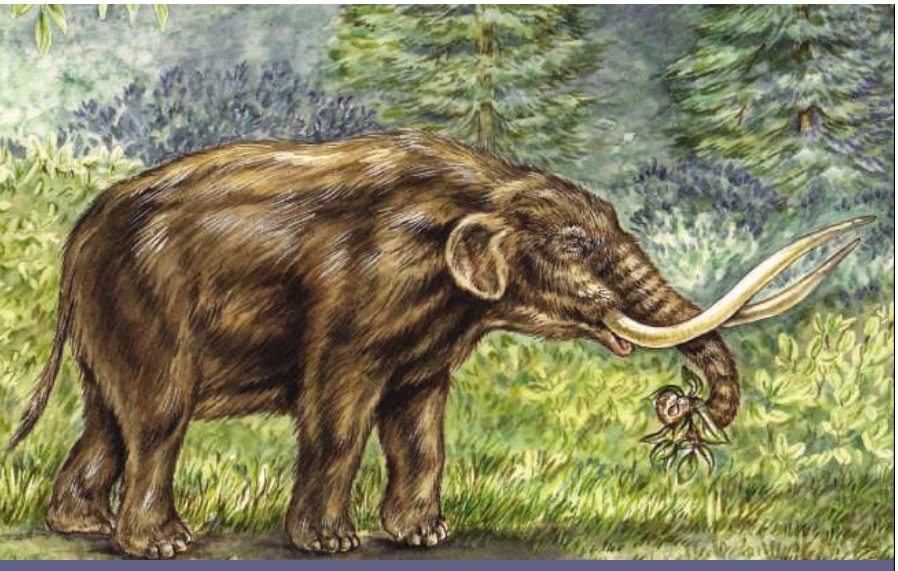
- Based on splinted-ligation technology — A bridge oligo hybridizes to a specific miRNA and a labeled detection oligo
- miRNA becomes directly labeled by ligation, then visualized

Benefits

- ***Speed*** - capture and label miRNA in just over 2 hours
- ***Sensitivity*** - detect miRNA in as little as 50 ng or less of total RNA
- ***Quantitative Results*** - accurate miRNA measurement in 6 hours

For more information on miRtect-IT™
call 800.321.9322 or visit www.usbweb.com/mirtect-it
In Europe: +49(0)76 33-933 40 0 or visit www.usbweb.de/mirtect-it

usb®
Fueling Innovation in Life Science™



WEB PROJECTS

RECALLED TO LIFE

Extinction isn't forever, at least on the Internet. After vanishing briefly, a popular fossil encyclopedia known as Palaeos has reappeared as a user-written wiki.* Paleontology buffs Toby Young and M. Alan Kazlev launched the trove of information on life's history 4 years ago, exploring topics as diverse as systematics techniques and the Cenozoic era (65 million years ago to the present), when this mastodon (above) stomped around North America. But Palaeos went offline this spring because it required too much work. Bloggers lamented, so Kazlev started a wiki. Users are invited to help transfer information from the old site and revamp the content. To browse the remains of the old Palaeos, click here.† >>
* www.palaeos.org † www.palaeos.com

RESOURCES

Bioinformatics for Dummies

Want a rundown on the differences between the UniProt, PROSITE, and PDB protein databases? Wondering how to find a paper that doesn't show up in PubMed? Try a session with the 2can primer from the European Bioinformatics Institute (EBI) in Hinxton, U.K. The site guides newbies and intermediate users who want a little help through a host of databases, online tools, and additional resources from EBI and other institutions. In the databases section, for example, you can learn when you might need PROSITE. It's useful for analyzing new proteins because it organizes molecules by characteristic sequences, or motifs, that can point to their functions. The tutorials pages offer detailed how-tos on several EBI programs. >> www.ebi.ac.uk/2can/home.html

TOOLS

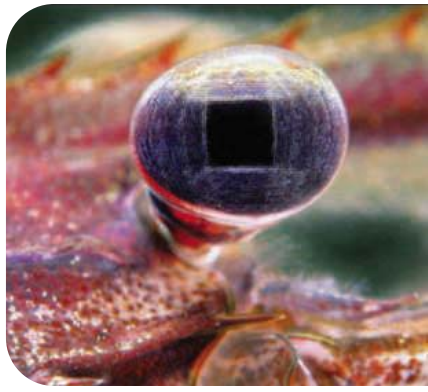
Weighing the Alternatives

Yield isn't everything when choosing how to synthesize an organic compound. Safety, cost of raw materials, the ease of isolating the products, and other considerations matter, too. To help evaluate these factors, fire up the calculator EcoScale, devised by chemists in Belgium, the United States, and Switzerland. Users key in the reactants, products, and details of the procedure, such as whether it requires specialized glassware or high pressure. EcoScale then rates each reaction from 0 to 100. Risky protocols reduce the scores by the largest amount. Explosive reagents blast 10 points off the final tally, for example. >> www.ecoscale.org

IMAGES

Time for a Close-Up

A freshwater shrimp (*Macrobrachium amazonium*) goggles at the camera through a square pupil (below). The shot by photographer Alex Griman of São Paulo, Brazil, nabbed one of the top places in this year's Small World contest, sponsored by the camera company Nikon. Held annually since 1974, the competition showcases photographic artistry through the microscope. Visitors can browse a gallery of this year's best submissions or check out previous winners. Peruse the backgrounders if you're curious about winning techniques such as stereomicroscopy, which Griman used to capture the shrimp image. >> www.nikonsmallworld.com/index.php



EXHIBITS

Building a Better …

Vannevar Bush (1890–1974) made magazine covers for directing the United States's scientific efforts during World War II. But he was also an inventor whose differential analyzer was a mechanical forerunner of the computer. At this site from the National Inventors Hall of Fame* in Akron, Ohio, you can read more about Bush and other scientists and engineers whose innovations had an impact. The hall inducts a new class of inventors every year. This year's honorees include the German chemist Fritz Haber (1868–1934), who discovered a method for making ammonia to produce fertilizer, and modern inventors such as Robert Gore, who created the waterproof fabric GORE-TEX. You'll find similar profiles at the Inventor of the Week† archive from the Massachusetts Institute of Technology. >>
* www.invent.org † web.mit.edu/invent/i-main.html

Send site suggestions to >>
netwatch@aaas.org
Archive: www.sciencemag.org/netwatch

CREDITS (TOP TO BOTTOM): LAURIE O'KEEFE/PHOTO RESEARCHERS INC.; ALEX GRIMAN; GETTY IMAGES

Hot Performance

Finnzymes' New Phusion™ Hot Start
The Number One DNA Polymerase

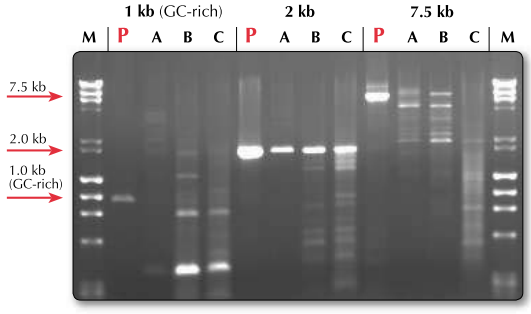
Accuracy The highest fidelity of any available thermostable polymerase

Speed Increased processivity allows reaction times to be reduced dramatically

Specificity Reduces non-specific amplification and primer degradation

Robustness Reduces reaction failures and minimizes optimization

Phusion - Extreme Performance



The following fragments were amplified from human genomic DNA: 1kb from CEBPB gene (GC-rich), 2 kb and 7.5 kb from human β-globin gene. Hot start high-fidelity DNA polymerases (P, A,B,C) were used according to suppliers' recommendations:

P) **Phusion™ Hot Start High-Fidelity DNA Polymerase**
A) novel *Pfu*-based fusion DNA polymerase
B) modified *Pfu* DNA polymerase
C) *T. kodakaraensis* DNA polymerase

Distributed in the US and Canada by New England Biolabs. For other countries visit www.finnzymes.com. PCR license notice: These products are sold under licensing arrangements of Finnzymes Oy with F. Hoffman-La Roche Ltd. The purchase of these products is accompanied by a limited license to use them in the Polymerase Chain Reaction (PCR) process in conjunction with a thermal cycler whose use in the automated performance of the PCR process is covered by the up-front fee, either by payment to Applied Biosystems or as purchased, i.e. an authorized thermal cycler.

FINNZYMES
TOOLS FOR MOLECULAR BIOLOGY

Finnzymes Oy • Keilaranta 16 A, 02150 Espoo, Finland • Tel. +358 9 584 121 • Fax +358 9 5841 2200 • fz@finnzymes.fi • www.finnzymes.com

A CASTLE FIT FOR A MATHEMATICIAN

Over the next few years, a full-scale replica of the Alhambra—the famed 14th century Moorish fortress in Spain—will rise in the bucolic hills south of San Jose, California. This summer, the city of Morgan Hill approved the building, which will house the American Institute of Mathematics (AIM). The castle is the brainchild of John Fry of Fry's Electronics, who in 1994 founded AIM and bought the 77-hectare grounds. For the past 9 years, as his dream—cost undisclosed—made its way past legal and technical hurdles, AIM has been running 24 seminars a year from a windowless warehouse in Palo Alto, California.

AIM Director Brian Conrey now expects the institute to move into its new 15,000-m^2 quarters by autumn 2009. Facilities will include a 150-seat lecture hall, a golf course, and eight gardens, including a reconstruction of Monet's garden in Giverny. The building's grandest space is reserved for a library with rare books and artifacts. Fry, who likes to model his stores on historical themes, "has talked for years about building this immense mathematical library—the equivalent of the Alexandrian library in the ancient world," says AIM board chair Gerald Alexanderson of Santa Clara University in California.

Polly Pachyderm

A couple of years ago, an elephant trainer at South Korea's zoo in Everland Resort outside Seoul thought he heard a human voice coming out of an elephant stall. The sounds turned out to be coming from one of his charges, Kosik.

Putting the end of his trunk into his mouth, the 15-year-old Indian elephant can say short words such as *bal* (foot), *joa* (good), and *anja* (sit). Elephants normally make sounds through their trunks, without using their mouths. Scientists believe that Kosik blows air out of his trunk, modifying its flow by aiming at different places in his mouth and thereby generating sounds through friction with molars, inner tusks, and tongue.

Zoo veterinarians and engineers from Soongsil University in Seoul have conducted tests with Kosik. The acoustical properties of the sounds he makes are similar to those of sounds made by his trainer, Jong Gap Kim. In effect, the scientists say, Kosik is acting like a parrot. Scientists plan to conduct further studies to find out how Kosik came to mimic his trainer. Veterinarian Yang Bum Kim says elephants, who are about as smart as human toddlers, are very group-oriented and tend to copy those closest to them, suggesting that Kosik has a strong bond with trainer Kim.

Kosik's parroting is not the first case of elephant mimicry. Last year, *Nature* published a paper on an African elephant that made "rumbling" sounds like a truck.

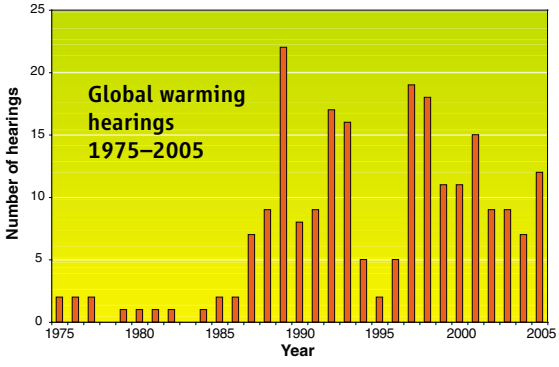
Kosik and trainer Kim.

Secondhand Radiation?

Scientists in Canada claim that trout exposed to x-rays can pass on the effects to nonirradiated fish.

Research in cell cultures has shown that low doses of ionizing radiation have "bystander" effects—causing damage to nearby, unexposed tissues. But there's been little evidence of this in live animals. A team led by radiation biologists Colin Seymour and Carmel Mothersill of McMaster University in Hamilton, Canada, theorized that this effect might also occur in fish, via the chemicals they release in the water. They gave a moderate dose of x-rays to eight rainbow trout swimming in a tank. They then put these fish in a tank with eight untreated trout and put eight other fish into the water that had held the irradiated fish.

After 2 hours, the scientists killed the fish. They found similar radiation effects in all three groups: Cells in several organs had died, and other cells were expressing proteins indicating radiation damage. The data suggest that bystander effects should be taken into account in assessing radiation risks to humans and animals, the team reports online 27 September in *Environmental Science & Technology*. Mary Helen Barcellos-Hoff, a biologist at Lawrence Berkeley National Laboratory in California, says it's possible "that such a phenomenon occurs between fish." But to prove it, the chemicals involved need to be identified.



<<AS EARTH WARMS, CONGRESS LISTENS

As the globe continues to heat up, the U.S. Congress holds more and more hearings that address the issue—including six last month. As of this week, according to the National Environmental Trust, Congress has held 233 hearings on global climate change since 1975. But although it's been authorizing more research, some complain that bold action to limit greenhouse gas emissions has been scarce. "Hearings don't do anything," says David Doniger of the Natural Resources Defense Council in Washington, D.C. "Legislation changes the marketplace, and that's what we need."

CREDITS (TOP TO BOTTOM): AIM; EVERLAND RESORT; SOURCE: NET

Advice on breaking ice 33

Physiology, physics Nobels 34 & 35

GENETICS

Seeing a 'Plot,' deCODE Sues to Block a DNA Research Center

A public brawl has broken out between deCODE Genetics, the Icelandic firm that created a country-wide DNA database to investigate diseases and develop drugs, and a U.S. hospital that recently announced the launch of the world's largest genotyping project focused on children's health. DeCODE has sued to stop the project, claiming it is built on information "stolen" by former deCODE employees.

"The defendants, led by Dr. Hákonarson and CHOP, plotted from at least September 2005 through May 2006 to **steal deCODE's most prized assets** and remove them to CHOP." —deCODE

In papers filed in a U.S. District Court and made public last week, the company accuses four employees of Children's Hospital of Philadelphia (CHOP) and one consultant—all ex-deCODE workers—of a "plot" to "steal deCODE's most prized assets" to create a commercial rival. Claiming that computer files and data were removed from Iceland, deCODE seeks an immediate court order to prevent CHOP from moving forward with its Center for Applied Genomics, a $39 million project to gather and analyze DNA from 100,000 children (*Science*, 16 June, p. 1584).

In a response filed with the Philadelphia court, CHOP denies the allegations and warns that if the court sides with deCODE, it could "paralyze … critical research into the genetic basis for childhood diseases." The court began a public hearing on the case on 26 September. No decision on an injunction had been announced as *Science* went to press, and some geneticists outside the fray are watching closely. Rory Collins of the University of Oxford, principal investigator for a massive new U.K. DNA bank, says of the CHOP project, "It would be a shame to lose a key resource such as this. It's hard enough to get the research started and approved; we need as many as we can get."

Officials at deCODE and CHOP have declined to comment on specifics of the lawsuit, but court documents indicate that the fight erupted after the deCODE employees moved to CHOP this summer. According to the legal complaint filed by deCODE, Hákon Hákonarson, an M.D.-Ph.D. and former deCODE vice president for business development, in September 2005 discussed a "business plan" with CHOP to create a large DNA data bank and genetics research center in Philadelphia. DeCODE alleges that Hákonarson negotiated a $100,000 "signing bonus" for himself and signed an employment letter with CHOP in December 2005, agreeing to head its fledgling DNA project. Hákonarson submitted his resignation in January and left deCODE in late May. However, the brief says, he did not fully inform deCODE's chief executive, Kári Stefánsson, about his intentions.

In the intervening months, according to deCODE, Hákonarson "on at least 60 occasions … exceeded his authorization to access deCODE's computer system by attaching removable storage devices" to obtain company research and business information that could be used to help set up CHOP's center. (DeCODE does not contend that any person's DNA sequences were copied.) "Dr. Hákonarson acted as a double agent for CHOP while appearing to remain a loyal employee," deCODE's brief alleges; it asserts that he recruited three other deCODE employees and a deCODE consultant to CHOP's payroll. In addition to seeking unspecified damages, deCODE asks the court to block the new Philadelphia center from competing with it for grants or business for at least 2 years.

"No software was taken. … deCODE's database of genetic information of the Icelandic population was not taken. … **No 'espionage' took place, and no trade secrets were 'stolen.'** " —CHOP

In its rebuttal brief, CHOP dismisses these "strident allegations" as "simply not true." The filing acknowledges that "some allegedly confidential information was innocently transferred to Dr. Hákonarson" at a time when "a proposed collaboration between deCODE and [CHOP] was in the making." But the information was "trivial," CHOP's brief says. The brief denies that CHOP's genetics research unit would be a commercial rival to deCODE.

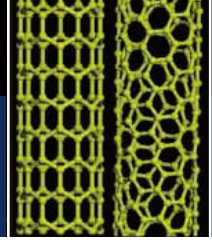
According to CHOP, relations soured when Stefánsson ordered Hákonarson's name removed from an "important" scientific paper. "Hákonarson had been considering leaving deCODE," the brief says, and told Stefánsson in January 2006 that he would resign—but at Stefánsson's request stayed on. Hákonarson and Stefánsson discussed the idea of deCODE and CHOP collaborating, according to the hospital's brief, but Stefánsson eventually rejected this and "fired" Hákonarson in May after becoming "angered over a review article Dr. Hákonarson published in a medical journal." CHOP blames the defection of deCODE employees on the firm's boss. "Because of Dr. Stefánsson's tempestuous behavior, the working environment at deCODE could be brutal," the CHOP brief says.

According to a deCODE statement, the company "believes it is in a strong position to secure its intellectual property and intends to do so." CHOP meanwhile asserts in a press release that "the claims against its researchers are without merit." **–ELIOT MARSHALL**

CREDITS (TOP TO BOTTOM): ROBERT GODDYN/UPA PHOTO; J. M. VINOCUR

NCI's new chief 40

Unraveling a cause of dementia 42



Cloning nanotubes 46

U.S. COMPETITIVENESS

Hopes for Innovation Bill Rest on Lame-Duck Congress

Members of Congress left town last week to campaign for reelection with little to show from a yearlong push for legislation aimed at bolstering U.S. competitiveness. But rather than being depressed, science advocates are hoping that the best is yet to come. The cause of their optimism: Senate leaders last week introduced a sprawling, bipartisan bill that would authorize $20 billion in new spending over 5 years to strengthen science and math education and expand federal research programs. Advocates hope the Senate will pass the measure, dubbed the American Competitiveness and Innovation Act, when Congress returns for a lame-duck session after the 7 November election. But persuading the House to approve a comprehensive innovation bill before the end of the year could be difficult. A different and much slimmer version has been languishing there for more than 3 months.

Last week, five influential lawmakers appealed to the community for help in getting the job done, appearing separately during a 1-day meeting at the National Academies that turned into an impromptu pep rally. "We need you to talk to your elected officials, and to the president, and tell them to support this bill," declared Senator Lamar Alexander (R–TN). The meeting marked the first anniversary of the publication by the academies of a widely acclaimed report—from a panel chaired by Norman Augustine and entitled *Rising Above the Gathering Storm*—that shaped the Senate bill (*Science*, 21 October 2005, p. 423).

It should be an easy sell, Senator Jeff Bingaman (D–NM) told the 600 university and corporate research leaders. "It's rare to get people on both sides of the aisle and on both sides of Capitol Hill to be singing from the same hymnal," said Bingaman, one of 32 co-sponsors of S. 3936, introduced 26 September by Senate Majority Leader Bill Frist (R–TN) and his Democratic counterpart, Senator Harry Reid of Nevada. "But that's the case with this topic and this bill."

"This bill contains almost all of the recommendations in the academies' report, and we're ready to move on it," proclaimed Senator Pete Domenici (R–NM), chair of the Energy and Natural Resources Committee. He proudly ticked off provisions that would authorize doubling the budget for the National Science Foundation over 5 years, doubling the budget for the Department of Energy's Office of Science over 10 years, and a slew of programs to train better science and math teachers and to attract more students into science, technology, engineering, and math fields.

A strong lineup. From left, NAS President Ralph Cicerone, Norman Augustine, NAE President Bill Wulf, and Senator Lamar Alexander enjoy the National Academies' convocation on competitiveness.

Then he added a caveat. "I also lead one of the appropriations subcommittees—Energy and Water—that has to fund a lot of what is in this bill, and we don't have money for everything. I have promised to do it by taking money from other areas. But I don't know if I will be able to hold on in upcoming negotiations with the White House and my colleagues. So I'm going to close my eyes and do my best."

Representative Sherwood Boehlert (R–NY), who chairs the House Science Committee, was equally candid about the hurdles in the House. Boehlert, whose retirement this fall after 24 years in Congress will further drain an already shallow pool of moderate House Republicans, has pushed unsuccessfully for floor debate on two bills that would expand existing research and education programs, H.R. 5356 and H.R. 5358, which in June cleared his committee. "Our bill includes a lot of what's in the *Gathering Storm* report," he said, "but, unfortunately, the bill has been stalled by a handful of conservative members who don't want to see any new spending and by some ideological forces in the White House. And the House leadership hasn't been willing to move it ahead." On the bright side, however, Boehlert said that the existence of a Senate bill, after months of wrangling over its content, improves the odds that "we will be able to work something out during the lame-duck session."

That postelection session poses its own problems. Beyond clearing must-pass spending bills and other legislation left over at the tail end of the 2-year congressional term, legislators must reconcile the House and Senate bills. Although they share the same party affiliation, Representative Boehlert and Senator Alexander don't exactly see eye to eye.

"I'd like to see a more streamlined version [of the 209-page Senate bill]. They need to set some priorities," says Boehlert. "The Senate bill doesn't have a prayer in the House. But I think we can pass something once we come to our senses."

For his part, Alexander predicts that S. 3936 "will go through the Senate quickly" once the Senate reconvenes on 14 November. "Then, the House could just pass our bill, and we'd be done." He also offered some not-too-subtle advice about winning over House Speaker Dennis Hastert (R–IL), whose district includes Fermi National Accelerator Laboratory.

"Suppose one of you lives in a district that contains a national lab and which happens to be represented by the Speaker of the House," Alexander said to laughter from the audience. "Maybe you could make a call."

–JEFFREY MERVIS

CREDIT: MARTY KATZ

A click of your mouse delivers over 1000 of ours.

Instant access to over 1,200 knockout mouse lines.

Taconic, one of the world's leading breeders of laboratory mice, and Lexicon Genetics, a pioneer in knockout mouse technologies, team up to bring you immediate access to an ever-expanding database of over 1,200 mouse models. Concentrated in the "druggable" gene classes, most knockout models already exist as live mice and can be delivered within weeks. Result? Readily available, immensely valuable research tools that accelerate the drug discovery and development process—and give you a serious competitive advantage.

The search ends here.

Visit our website **http://taconic.lexgen.com**
to download the entire portfolio or search for a gene of interest.

Taconic

POLAR RESEARCH

U.S. Needs New Icebreakers, Report Tells Congress

Despite the United States's growing economic, military, and scientific interests in the polar regions, for the past 2 years the National Science Foundation (NSF) has relied on a rented Russian icebreaker to make sure that the U.S. research stations in Antarctica don't run out of fuel. The need to have a former Cold War archrival protect U.S. strategic interests is more than simple irony, however. A new report by the National Academies says that it's unacceptable—and that the federal government should build and deploy two new icebreakers in the next decade to make sure that the United States has full and timely access to the region. But finding the political will—and the money—to get the job done is another matter.

The report, from a panel of the Polar Research Board that was chaired by computer engineer Anita Jones of the University of Virginia in Charlottesville, scolds the government for allowing the current four-ship U.S. icebreaking fleet to deteriorate and recommends that the Bush Administration review its recent decision to shift management and fiscal responsibilities for icebreaking from the Coast Guard, where they have historically resided, to NSF, which coordinates U.S. polar research and runs the Antarctic program (*Science*, 16 December 2005, p. 1753). Jones says the $1.4 billion cost of two new ships is a small price to pay to ensure U.S. access to both polar regions, noting for example that accelerated melting of sea ice has focused new global interest on the Arctic.

"I'm concerned about the diminishing capacity of our current fleet," says Representative Don Young (R–AK), chair of the House Transportation Committee, whose Coast Guard and maritime subcommittee held a hearing last week on the academies' report. The Alaskan legislator, who had requested the report, noted that global warming presents an unprecedented opportunity for the United States to develop shipping lanes and natural resources in the Arctic, and that he's been frustrated by the inaction of the Bush Administration in renovating the icebreaking fleet: "Maybe this report will help."

One major issue for the panel is the country's ability to maintain open water each winter for oil tankers to resupply McMurdo Station, the largest of the three U.S. stations on the Antarctic continent and the staging area for activity at the South Pole. The mainstays of the U.S. fleet are the *Polar Sea* and *Polar Star*, the world's most powerful nonnuclear icebreakers. Both are now at the end of their expected 30-year life spans. Their increasing fragility, combined with unusually thick pack ice in the Antarctic, forced NSF to rent the Russian icebreaker *Krasin* for several weeks during the past two winters. (The Coast Guard also operates the *Healy*, a 7-year-old research icebreaker used mainly for Arctic missions, and NSF leases the *Nathaniel B. Palmer*, which has limited icebreaking capabilities, for Antarctic research cruises.) NSF has lined up a Swedish icebreaker for this winter's Antarctic chores.

With a little help … Russian icebreaker *Krasin* clears path to NSF base.

CREDIT: PATRICK ROWE/NATIONAL SCIENCE FOUNDATION

"Congress likes to wait until there's a crisis, and unfortunately, that's what it has come to," says Representative Frank LoBiondo (R–NJ), chair of the maritime subcommittee. Mead Treadwell, chair of the U.S. Arctic Research Commission and a witness at the hearing, hopes the report will help the scientific community pressure the Bush Administration to address the issue. "The first step would be $30 million in the [president's 2008 budget request] for a design study," says Treadwell. "If it's not there, then we'll have to start screaming."

–JEFFREY MERVIS

Mars (Sand) Bars

Now that the Opportunity rover has reached the edge of Victoria Crater on Mars, its handlers have some major decisions ahead. "It's not abundantly clear Opportunity can get in," says rover science team leader Steven Squyres of Cornell University, "nor is it abundantly clear it can get out."

Opportunity once spent 5 weeks getting itself off a sand ripple, but the 21-month trek to Victoria made clear how few alternative study sites are nearby. So the rover will circle the crater rim in the coming months to help scientists balance potential science to be done against the hazards of negotiating steep, sandy slopes. The attitude of some? Damn the dunes, full speed ahead. **–RICHARD A. KERR**

Gravity Research Pulled Together

An agreement to share data could turn the world's gravitational-wave observatories into one big instrument. It would allow researchers working with the Virgo detector near Pisa, Italy, and their counterparts with the Laser Interferometer Gravitational-Wave Observatory (LIGO) in the United States to share data and jointly publish all results, says Benoit Mours, Virgo spokesperson and a physicist at the Annecy-le-Vieux Particle Physics Laboratory in France. LIGO has already joined forces with GEO 600, a smaller gravitational-wave detector near Hannover, Germany. The new deal must still be approved by the Italian and French agencies that fund Virgo, but Mours says he's confident that will happen.

–ADRIAN CHO

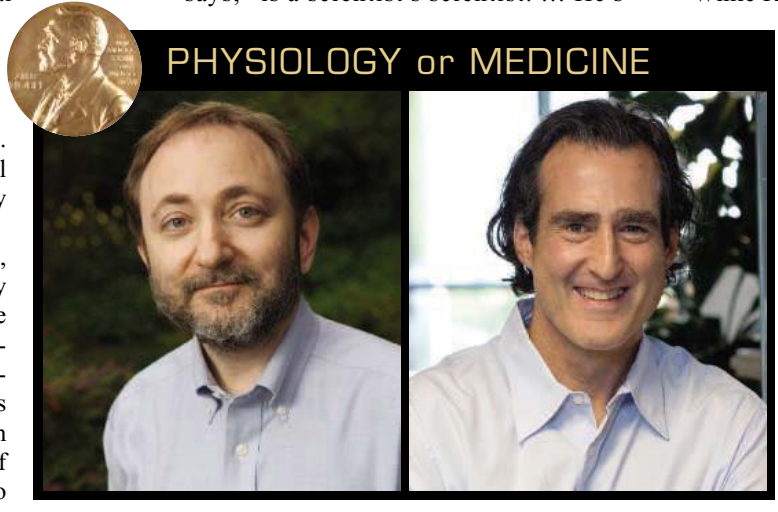
In the Crosshairs: Pork

Last week, the U.S. Congress put off until November a move to increase transparency on earmarks, the controversial projects that lawmakers write into spending bills without peer review or merit competitions. Universities, often the recipients of earmarks for research, were unhappy when spending hawk Senator Tom Coburn (R–OK) queried schools on the practice in a probe last month (*Science*, 8 September, p. 1374). Last week, however, a House-Senate conference stripped language from a military spending bill Coburn wrote that would have required the Pentagon to make its earmarks details public. So Coburn threatened to hold the important bill up for final vote, winning a promise that Congress would revisit the issue in a November lame-duck session. "The American people should not have to obtain a search warrant to know how we are spending their money," says a Coburn spokesperson. **–ELI KINTISCH**

NOBEL PRIZE: PHYSIOLOGY OR MEDICINE

Method to Silence Genes Earns Loud Praise

Over beer and coffee, in labs and at scientific conferences, the speculation has been intense for years: Who in the RNA interference (RNAi) field, biologists wondered, would win the Nobel Prize, and when? Science's ultimate accolade was considered increasingly inevitable as the gene-silencing method revolutionized genetics, spurred development of new medical treatments, and transformed our understanding of cellular behavior. But, under Nobel rules, the prize can go to no more than three people. Yet many had made seminal contributions to the discovery and understanding of RNAi.



Silence is golden. Andrew Fire (*left*) and Craig Mello learned this week that they'd won the Nobel Prize for their groundbreaking discovery of RNAi's gene-quelling power.

Early Monday morning, several years earlier than many expected, the guessing game came to an end. Two Americans—Craig Mello of the University of Massachusetts (UMass) Medical School in Worcester and Andrew Fire of Stanford University in Palo Alto, California—learned that they had won this year's $1.37 million Nobel Prize in physiology or medicine. Like many Nobel winners before him, Fire, who was woken by a phone call from Sweden, wondered at first if he was dreaming, or the caller had the wrong number. Although many had predicted that he and Mello would be winners, Fire still felt a "certain amount of disbelief," he said during a press conference. "We looked at this very, very complex jigsaw puzzle and put in a significant piece," he said.

That piece came in 1998, when the pair, with colleagues, reported in *Nature* that injecting double-stranded RNA into worms silenced genes. That nailed down the mechanism for seemingly disparate and baffling observations others had made in plants, worms, and even mold over previous years. It also laid the groundwork for subsequent RNAi findings, including the discovery of the phenomenon's natural roles in mammalian cells—guiding early development, for example—and ways to manipulate it artificially. Today, it's thought that one type of RNA, microRNA, depends on RNAi to control upward of a quarter of the human genome.

Although Fire said at a press conference that, given the strides made by others in the field, "I feel slightly guilty to be here," Phillip Zamore, who works with Mello at UMass, calls the award "one of the most well-deserved Nobel Prizes ever given." The *Nature* paper, Zamore notes, prompted him to leap into the RNAi field at the end of his postdoc 8 years ago, much as it inspired many other young researchers. Mello, he says, "is a scientist's scientist. … He's always stretching me intellectually."

Adds Phillip Sharp of the Massachusetts Institute of Technology in Cambridge, who himself won a Nobel 13 years ago for RNA discoveries and now works with RNAi, "The avalanche was started down the hill by this paper."

Fire, who at the time was based at the Carnegie Institution of Washington in Baltimore, Maryland, and Mello resolved the mystery of RNAi while experimenting with ways to control levels of a muscle protein in worms by ramping up or down its RNA. Like others, they recorded puzzling results when injecting either "sense" or "antisense" RNA into cells. These single strands of RNA were thought to either boost or reduce levels of messenger RNA that had matching or complementary sequences, but they did not act predictably. When Fire and Mello combined the strands of RNA into a double-stranded helix, they were taken aback by its power to dampen gene expression in their worms reliably and specifically.

Several years later, Thomas Tuschl, who is now at Rockefeller University in New York City, translated the findings to mammalian cells. Dozens of companies are now seeking to apply RNAi to medical conditions as diverse as cancer and hepatitis, using the method to turn off oncogenes or shut down viral replication, for example. Meanwhile, labs around the world routinely test the function of proteins in cells or generate animal models of diseases by using RNAi to silence genes.

While RNAi researchers praised Mello and Fire's experiments and deemed them a key turning point for the field, some also voiced quiet disappointment that the committee had decided not to share the prize with a third person. One RNAi researcher, who asked not to be named, even told *Science* that "plants got screwed."

It was observations in plants that first hinted at a mysterious gene-silencing method. But the findings were puzzling, disparate threads that never quite tied together. In the early and middle 1990s, researchers such as David Baulcombe of the Sainsbury Laboratory in Norwich, U.K., determined that adding genes into plants sometimes turned off the endogenous counterparts, a phenomenon then described as cosuppression (*Science*, 26 May 2000, p. 1370). Baulcombe and others suspected that RNA was behind this gene silencing but were unable to control the outcome of their experiments reliably. Around the same time, Victor Ambros of Dartmouth Medical School in Hanover, New Hampshire, and Gary Ruvkun of Harvard Medical School in Boston identified in worms a natural gene-silencing RNA that would later become known as microRNA.

At a plant conference in 1995, Ruvkun discussed his worm work, recalls Richard Jorgensen, a plant geneticist at the University of Arizona, Tucson, who made some of the earliest discoveries of RNAi. "We talked about it, and we kind of shrugged our shoulders. We couldn't figure out what the connection was," he says.

It took Mello and Fire's results with double-stranded RNA for that light bulb to go on. Baulcombe, who many cited as deserving Nobel recognition, too, graciously acknowledged that, calling the pair's work "immensely significant." **–JENNIFER COUZIN**

CREDITS (LEFT TO RIGHT): THE NOBEL FOUNDATION; LINDA A. CICERO/STANFORD NEWS SERVICE; UNIVERSITY OF MASSACHUSETTS MEDICAL SCHOOL

NOBEL PRIZE: PHYSICS

Astrophysicists Lauded for First Baby Picture of the Universe

The 2006 Nobel Prize in physics honors two astrophysicists who first mapped the afterglow of the big bang—the so-called cosmic microwave background (CMB). Using an instrument on NASA's Cosmic Background Explorer (COBE) satellite, John Mather, 60, of NASA's Goddard Space Flight Center in Greenbelt, Maryland, and colleagues measured the precise spectrum of the microwaves. Using another instrument on COBE, George Smoot, 61, of Lawrence Berkeley National Laboratory and the University of California, Berkeley, and colleagues detected slight variations in the temperature of the CMB, signs of the clumping of matter that would produce galaxies.

The portrait of the universe as a young fireball paved the way for today's studies of how the cosmos evolved, says George Efstathiou, an astrophysicist at the University of Cambridge in the U.K. The spectrum "verified beyond any reasonable doubt that the cosmic microwave background radiation was created very early in the universe's history," he says, and "the discovery of temperature ripples gave us the first probe of the exotic physics that occurred within 10^{-35} seconds after the big bang."

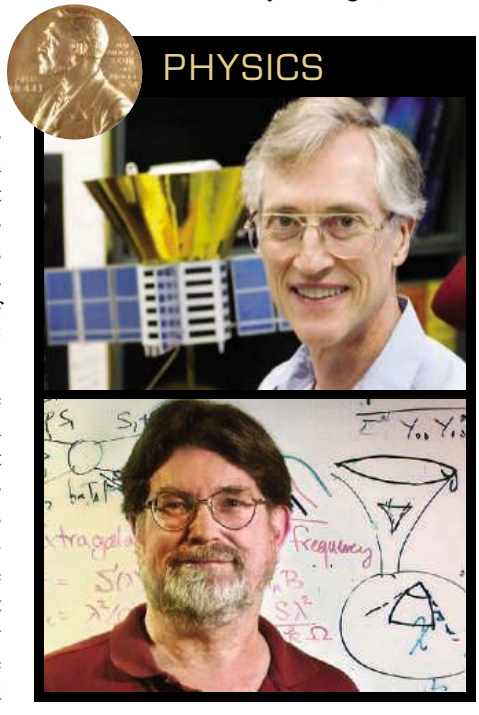
According to the big bang theory, the universe burst into existence and has been expanding and cooling ever since. Almost 14 billion years later, radiation from the primal explosion lingers, although it has cooled to a frigid 2.725 kelvin as its wavelength has stretched to the microwave range. Physicists Arno Penzias and Robert Wilson discovered those microwaves by accident in 1965 and won the Nobel Prize 13 years later. But when NASA launched COBE in 1989, scientists were still puzzling over the CMB's properties.

Mather's team showed that the spectrum of the radiation fit a so-called blackbody spectrum that describes a glowing body in thermal equilibrium. That meant that the radiation was released quickly and that the early universe was very hot and dense, as the big bang theory predicted. "In the beginning, I was just trying to get the job done, and I didn't think about how important [the measurement] was," Mather says. "But since then, I've realized that it is an essential piece of the history of the universe."

Smoot's team found that the temperature of the radiation varied from place to place in the sky by about one part in 100,000—just enough to be detected. "It was close," Smoot says. "We had about a factor of 2 margin" in sensitivity. The small size of the variations indicated that the gravity from ordinary matter would not suffice to explain the structure of the universe; some form of unobserved dark matter must have sown the seeds for the galaxies and clusters, Smoot says.

Mather and Smoot were postdocs in the 1970s when they proposed their experiments. "NASA was taking a big chance on us," Smoot says. Since COBE produced its first results in 1992, the CMB has borne still more fruit. Three years ago, NASA's Wilkinson Microwave Anisotropy Probe satellite obtained a more-detailed map, which revealed the precise age and composition of the universe. And next year, the European Space Agency will launch Planck, a satellite that will map the polarization of the microwaves, which could reveal signs of gravitational waves in the primordial universe. "I think there [are] many things left to learn about the CMB," Mather says. And perhaps more Nobels to come. **–ADRIAN CHO**

With reporting by Daniel Clery.



First light. Astrophysicists John Mather (*top*) and George Smoot and colleagues probed the details of the afterglow of the big bang.

CREDITS (TOP TO BOTTOM): NASA; ROY KALTSCHMIDT/LBNL

Cell Work Halted …

AMSTERDAM—A clinic in the Netherlands was ordered to stop administering controversial treatments with umbilical cord blood cells on Monday after an investigation concluded that the $23,000 injections may expose patients to serious risks.

The Preventive Medicine Center (PMC) in Rotterdam was one of several clinics and companies around the world offering stem-cell treatments that many mainstream scientists and physicians consider unproven and potentially harmful (*Science*, 14 July, p. 160). In a letter, the Dutch Health Care Inspectorate concluded that PMC cannot document the "origin, suitability and safety" of the cells it injects, and that as a result, patients risk being infected with HIV, hepatitis, and Creutzfeldt-Jakob disease and developing tumors. One patient had to be hospitalized recently with acute allergic reactions, the letter noted.

"This is justice, finally," says neurologist Rogier Hintzen of Erasmus Medical Center in Rotterdam, who had prodded the inspectorate to take action. PMC did not return calls requesting comment. The investigation into a second Dutch stem-cell company, Cells4Health, is continuing, an inspectorate spokesperson says. **–MARTIN ENSERINK**

… And Sought

Wisconsin has taken a new step to lure stem-cell science to the Badger State. Last week, the Wisconsin Alumni Research Foundation (WARF)—which holds the U.S. patents on human embryonic stem cell technology—announced that it will no longer charge licensing fees, which range from $75,000 to $400,000, to companies in the state that sponsor research at universities and other nonprofit organizations. WARF says only two Wisconsin companies currently do such research.

Governor Jim Doyle announced the policy as part of an economic development package that includes grants of up to $250,000 for stem-cell companies that move to the state. Doyle is in a tight election race with Republican Mark Green, who supports work on existing lines but opposes work on stem cells created by destroying embryos.

Jerry Flanagan, head of the California-based Foundation for Taxpayer and Consumer Rights, which has filed administrative challenges on WARF's patents (*Science*, 21 July, p. 281), says the new policy "is an acknowledgment that the overly broad WARF patents stymie research and delay cures."

–CONSTANCE HOLDEN

ASTRONOMY

Speedy Planets Near Galactic Center Show Sun's Region Is No Fluke

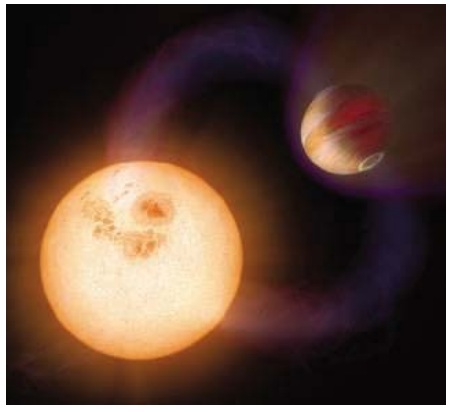
The planet orbiting the dwarf star SWEEPS-10 is probably lifeless. But if you were born there, you would celebrate your "birthday" every 10 hours and 11 minutes. That's all the time it takes the Jupiter-like planet to complete one revolution. In addition to smashing the old record for shortest-period planet, SWEEPS-10 B—along with 15 other new exoplanets discovered by the Hubble Space Telescope—gives astronomers the first evidence that extrasolar planets are just as abundant in the center of the Milky Way galaxy as they are in the neighborhood of our sun.

A team led by astronomer Kailash Sahu of the Space Telescope Science Institute in Baltimore, Maryland, used Hubble to monitor 180,000 extremely faint dwarf stars in the core of the Milky Way, some 30,000 light-years away. Most of the 200 or so previous exoplanets were spotted by periodic Doppler shifts: changes in the wavelength of their stars' light that result from stellar wobbles caused by a planet's gravitational pull. That method limited astronomers to nearby stars, the only ones bright enough to be studied spectroscopically. The new search was based on a different principle: If a star has a close-in planet with an orbit seen edge-on, the planet should periodically block a small part of the star's light. The results of the week-long Sagittarius Window Eclipsing Extrasolar Planet Search (SWEEPS), carried out in February 2004, are published in this week's issue of *Nature*. The haul of 16 planet candidates includes five that orbit their stars in less than an Earth day.

"It's a very tantalizing result," says astronomer Don Pollacco of Queen's University Belfast in the U.K., "but you have to be cautious. These stars are so faint that it's incredibly tough to do follow-up observations" to prove that the brightness dips are indeed caused by transiting planets and not by binary companion stars or other effects. Pollacco is the project scientist of SuperWASP, a ground-based search for transiting exoplanets that recently published its first two detections.

SWEEPS's five ultrashort-period planets all seem to orbit stars that are significantly less massive than the sun. Because low-mass stars are also cooler and less luminous, the new exoplanets are about the same temperature (some 2000 kelvin) as the many "hot Jupiters" that have been found at slightly larger distances from brighter, hotter stars. Planet hunter William Cochran of the University of Texas, Austin, speculates that 2000 K may mark the highest temperature a "gas giant" planet can reach before its atmosphere evaporates, leaving only a small, rocky core.

In the near future, two space missions will also look for transiting exoplanets. The French COROT satellite is scheduled for launch in late November this year, followed by NASA's Kepler mission in 2008. Cochran, who is a co-investigator on Kepler, says the Hubble results are "of significant importance" for the space missions, because they seem to confirm that the statistics of extrasolar planets are the same throughout the galaxy. **–GOVERT SCHILLING**



Playing with fire. Record-setting exoplanets may show how close to a star "gas giants" can orbit and live to tell the tale.

Govert Schilling is an astronomy writer in Amersfoort, the Netherlands.

GEOCHEMISTRY

Has Lazy Mixing Spoiled the Primordial Stew?

Last year, geochemists working out how the solar system was cooked up 4.6 billion years ago thought they had found some missing ingredients. A slightly skewed isotopic composition of meteorites suggested that some elements had been dragged into the deep Earth early on, in zones of rock that remain undetected to this day. That "layer cake" model of Earth's interior solved a number of problems for terrestrial geochemists. But two more isotopic studies published online by *Science* this week (www.sciencemag.org/cgi/content/abstract/1131708 and www.sciencemag.org/cgi/content/abstract/1132595) indicate that the notion of permanent layering in Earth's depths may rest on shaky assumptions about the chemistry of the early solar system.

The new papers agree that some isotopes vary in abundance among meteorites (and thus among the asteroids they came from), or between meteorites and Earth. Those variations imply that isotopic composition varied from place to place in the swirling disk of gas and dust that gave rise to planets and asteroids. Last year's paper, by contrast, assumed that the solar nebula was thoroughly mixed (*Science*, 17 June 2005, p. 1723).

That paper's authors, geochemists Richard Carlson of the Carnegie Institution of Washington's Department of Terrestrial Magnetism and Maud Boyet, now at University Jean Monnet in St. Etienne, France, found a previously unrecognized difference in the isotopic composition of the element neodymium between meteorites and rocks from Earth. The meteorites were so-called chondrites, little-altered building blocks of the rocky planets. Geochemists have ▶

A planetary mixing bowl. Geochemists had assumed that the swirling solar nebula thoroughly stirred in all the planetary ingredients, but meteoritic isotopes now show the mixing was incomplete.

CREDITS (TOP TO BOTTOM): NASA/ESA/A. SCHALLER; NASA/JPL-CALTECH/T. PYLE (SSC)

long thought they have the same composition as Earth's rock. So Boyet and Carlson assumed that the newly recognized neodymium gap arose soon after Earth formed, as a result of chemical reactions that separated samarium from other elements. Samarium is a radioactive progenitor of neodymium.

But in one of this week's papers, geochemists Michael Ranen and Stein Jacobsen of Harvard University report that the solar nebula was isotopically heterogeneous in the first place. The evidence comes from barium, one of the elements that swirled into the solar nebula after being forged by nuclear reactions in a dying star. The authors find more of some barium isotopes in chondritic meteorites than in rocks on Earth even though the barium isotopes—unlike the neodymium isotopes—are not the products of radioactive decay. Somehow, they say, some of the newly minted elements did not get thoroughly mixed into the nebula before the asteroids formed. As a result, the chondritic meteorites cannot be trusted as a benchmark for the starting composition of the whole Earth, they conclude, contrary to Boyet and Carlson's assumption. Earth's initial composition "becomes a more complicated puzzle to figure out," Jacobsen says.

The authors of the second online paper—geochemists Rasmus Andreasen and Mukul Sharma of Dartmouth College—also found signs of a heterogeneous solar nebula, but with a twist. They revisited the neodymium and samarium isotopes of chondritic meteorites. They found that the most primitive sort, the carbonaceous chondrites from the far edge of the asteroid belt, contain a mix of neodymium isotopes different from that in ordinary chondrites from the inner part of the belt.

Carbonaceous chondrites are definite oddballs, Andreasen and Sharma conclude. But they see signs in neodymium and samarium isotopes that Earth and ordinary chondrites grew from the same sort of stuff. The difference in neodymium isotopes that Boyet and Carlson noted could indeed have resulted from the early separation of elements on Earth, they say. Andreasen and Sharma's analyses "bolster our claim" about a layered deep Earth, says Carlson. Sharma agrees.

The two new isotopic studies agree in one respect. "What was supposed to be a homogeneous stew was not," says geochemist Gerald Wasserburg, professor emeritus at the California Institute of Technology in Pasadena. "I don't know whether Boyet and Carlson are right or not, [but heterogeneity] threatens all the things one does in that area. Pandora's box is clearly open."

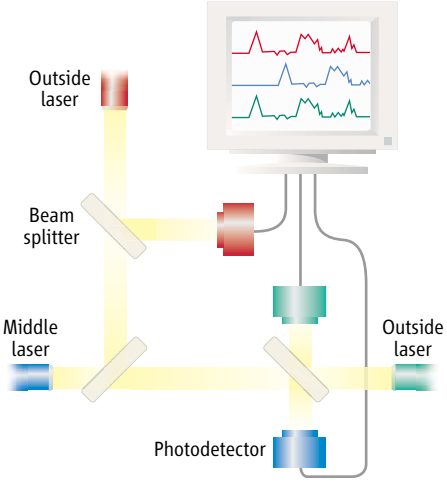
–RICHARD A. KERR

ILLUSTRATION: P. HUEY/*SCIENCE*

NONLINEAR DYNAMICS

Bizarrely, Adding Delay to Delay Produces Synchronization

Running late? Add more delay, and you can end up right on time—if you happen to be a chaotically varying beam of laser light. When three lasers in a row shine into one another in just the right way, they can forge a connection in which the intensities of the first and last lasers vary in unison, physicists report. That's weird because if the researchers couple only two lasers, the variations of one simply lag those of the other by the amount of time it takes light to pass between them, as anyone might expect. The strange new effect could shed light on how the hemispheres of the brain stay in sync, researchers say.



Tag team. When three lasers couple, the outer two (red and green) stay in sync as the middle one lags.

"These guys have shown experimentally that this happens," says Rajarshi Roy, a physicist at the University of Maryland, College Park. "Explaining mathematically why this is possible is an open question."

When two lasers shine into each other, their intensities can start to vary randomly. The heart of each laser is a "resonant cavity" in which light begets more light in a process called stimulated emission. Within one laser, light from the other laser can interfere with the light already in the cavity, either increasing or decreasing the overall intensity. That change, in turn, increases or decreases the output of the laser and hence the amount of light beaming back into the other one. Such feedback can trigger chaotic oscillations in the intensities of both.

Ingo Fischer of the Free University of Brussels, Belgium, and colleagues previously had shown that when two lasers couple, the fluctuations in one always lagged the other. But when the researchers added a third laser to the chain—so that the lasers on the ends shone into the one in the middle and the one in the middle shone into those on the ends (see diagram)—they got a surprise. The laser on one end instantaneously reproduced the variations of the laser on the other end, even as the middle laser trailed behind by 3.65 nanoseconds, the time it took light to travel the 1.1 meters between neighboring lasers, the team reports in the 22 September *Physical Review Letters*.

The effect might conjure up thoughts of faster-than-light communication, but that's not possible, Fischer says. The random variations are produced by the system as a whole, so it is impossible to feed a message into one end of the chain and immediately extract it from the other, he says.

Although it may not challenge the laws of physics, the experiment could help decipher the synchronization of nerve signals in the brain, says Wolf Singer, a neuroscientist at the Max Planck Institute for Brain Research in Frankfurt, Germany. In 1986, Singer and colleagues showed that networks of neighboring neurons tend to fire at the same time, and 5 years later they showed that such tight synchrony extends to the opposite hemispheres of the brain—even though it takes 6 to 8 milliseconds for nerve impulses to propagate that far.

Such synchronization may help define individual neural circuits, Singer says, and researchers can already explain how local networks of neurons get in sync. "What is less well understood is how remote sites get synchronized," Singer says, "and that's where this work may be relevant."

Analyzing the effect may not be easy, says Jürgen Kurths, an expert in nonlinear dynamics at the University of Potsdam in Germany. Without the delays, the coupled lasers can be described with a finite number of equations. Add the delays, and "in theory you have an infinite number of equations, so it becomes quite difficult," Kurths says. Understanding will come, he says, but it may take time.

–ADRIAN CHO

And the 2006 winner is...

Doris Tsao, Ph.D.

University of Bremen, Germany

$25,000 Prize

Congratulations to Dr. Doris Tsao on winning the 2006 Eppendorf & *Science* Prize for her work in determining the neural basis for face perception. Dr. Tsao's findings represent the first direct demonstration that a dedicated brain area, consisting almost entirely of face cells, exists to carry out face processing. Systematically probing how these face cells vary their responses to changes in facial form should allow us to understand how a complex form such as a face is encoded by single neurons.

The annual $25,000 Eppendorf and *Science* Prize honors young scientists for outstanding contributions to neurobiology research. Dr. Tsao is the fifth recipient of this prestigious award, and she will be honored at a ceremony held during the week of the 2006 Annual Meeting of the Society for Neuroscience.

You could be next.

If you have received your Ph.D. or M.D. within the past 10 years, you may be eligible to win the 2007 Prize. Entry deadline is June 15, 2007.

For more information: **www.eppendorf.com/prize or www.eppendorfscienceprize.org**

"I feel incredibly honored to receive this prestigious prize."

2006 Winner
Doris Tsao, Ph.D.
Institute for Brain Research
University of Bremen
Germany

eppendorf & Science PRIZE FOR NEUROBIOLOGY

FRANCE

Presidential Hopefuls Discover a New Issue: Science

FLEURANCE (GERS), FRANCE—French scientists can barely believe it. In the run-up to the April-May 2007 presidential elections, research appears to be shaping up as a serious issue. The evidence: A parade of presidential hopefuls traveled to this tiny village 700 kilometers south of Paris last weekend—joining a retreat of the movement known as Sauvons la Recherche (SLR)—to engage scientists in debate and promise them better times.

True, the two hottest candidates were absent. Nicholas Sarkozy, the populist minister of the interior and the frontrunner in the conservative party UMP, had declined the invitation. Media darling Ségolène Royal, the leading Socialist Party (PS) candidate—who would be France's first female president if elected—agreed to come months ago but canceled at the last minute, angering some of the more than 400 participants.

On the stump. François Bayrou was one of seven presidential candidates who sought support at a meeting of researchers last week.

But that left seven candidates, spanning the political spectrum from the Revolutionary Communist League—a Trotskyist group whose candidate, Olivier Besancenot, is a 32-year-old mailman—to the centrist Union for French Democracy (UDF). The result was a lively, occasionally raucous exercise in democracy, with most candidates fielding tough questions for almost an hour each while they were mocked real-time by a cartoonist whose drawings were projected on a giant screen behind them.

Politicians have reason to court scientists this year. An SLR-led revolt against budget cuts and poor prospects for young researchers brought tens of thousands to the streets and may have helped defeat the governing UMP in regional elections in 2004. In response to the uprising, President Jacques Chirac offered a research reform bill, dubbed the "Pact for Science," which the National Assembly approved this spring. Touted as an unprecedented shot in the arm for French science, the bill raises the overall research budget about 20% and creates thousands of new jobs. But SLR, which objected to some elements such as the new National Research Agency, says the bill falls short of what's needed (*Science*, 10 March, p. 1371).

The candidates who came to the meeting agreed—and they knew how to flatter. "You are one of the two or three keys to the future of France," said UDF leader François Bayrou, adding that if elected, he would not only offer a 10-year investment program including 5% annual budget growth but also boost the image of science in French society. "Your community has been the victim of unjust and deleterious measures," said former prime minister and PS candidate Laurent Fabius, who proposed the most detailed package, including a 10% annual budget increase and a program to boost the life sciences, with emphasis on stem cells, antibiotics, and DNA tests.

The pols did not escape criticism, however. Biologist and former SLR spokesperson Alain Trautmann argued that PS heavyweights such as Fabius and Royal could have done more to improve the science bill during debate on it. And Dominique Voynet, the candidate for the Greens, came under fire for her party's hostility toward nuclear energy and genetic modification. But in the end, the audience liked most of what it heard.

Whether the researchers will make a difference in the election is anything but certain. Except for Fabius, none of the candidates has a real shot at the presidency, and in the end, science will likely remain an electoral sideshow compared to issues such as the economy, joblessness, and immigration, concedes physicist and SLR president Bertrand Monthubert. Still, the fact that politicians are paying attention is an important step in the right direction, he says: "We have never had an opportunity like this before." **–MARTIN ENSERINK**

BIODEFENSE

House Passes Plan for Drug, Vaccine R&D

Hoping to plug holes in the nation's biodefenses, the U.S. House of Representatives last week approved a plan to create a new body to coordinate and finance the development of drugs and vaccines.

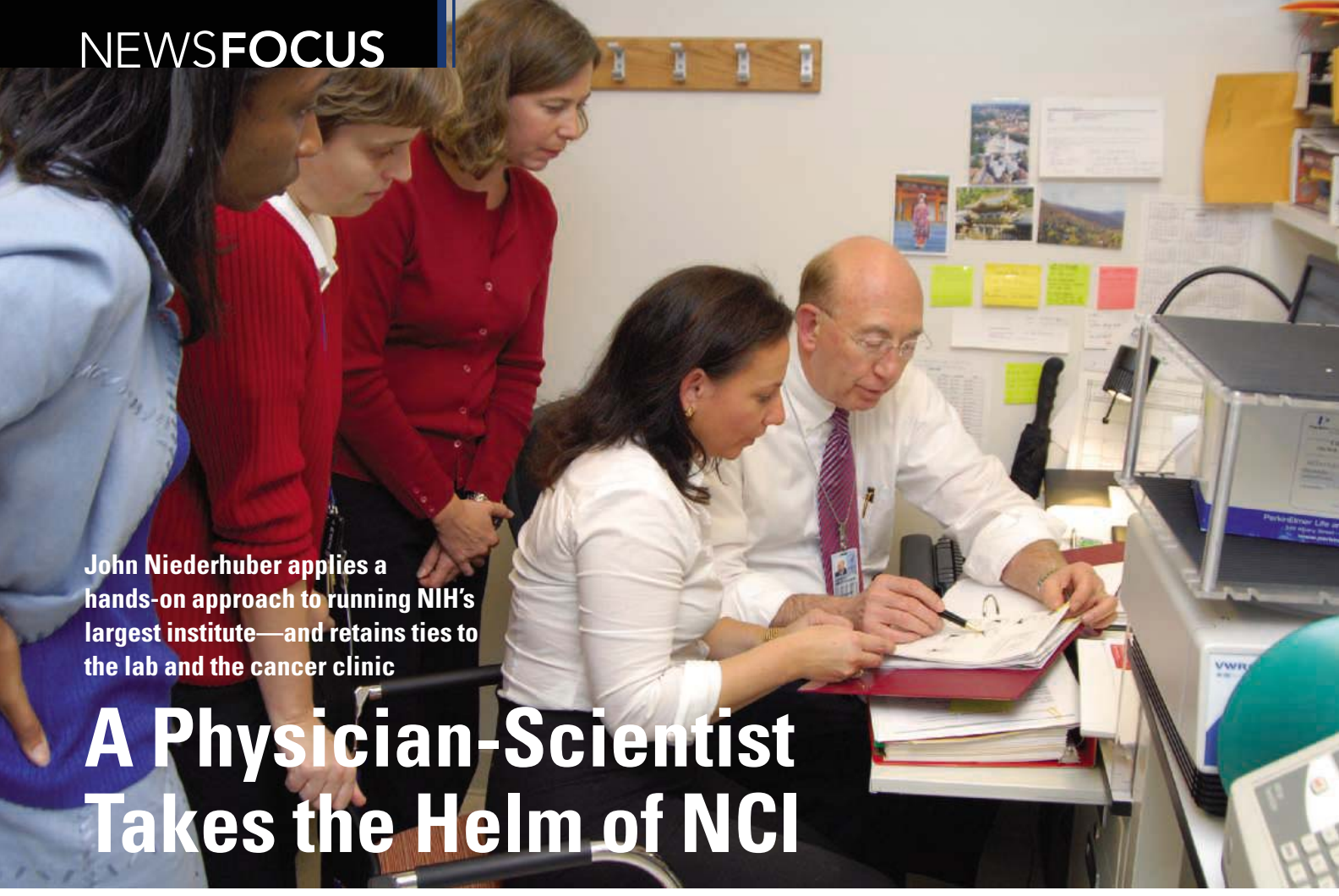
The measure is meant to improve on Project BioShield, a $5.6 billion procurement fund that critics say has been slow to add new countermeasures to the nation's stockpile (*Science*, 7 July, p. 28). H.R. 5533 creates a Biomedical Advanced Research and Development Authority (BARDA) within the Department of Health and Human Services (HHS) to oversee and fund the development of defenses against bioweapons and natural outbreaks. The bill authorizes $160 million a year in 2007 and 2008 to help companies through the "valley of death"—manufacturing scale-up and clinical trials—before applying to BioShield. It sets up an advisory board with industry members to help identify new threats, and it allows BioShield payments when companies meet "milestones" instead of only when they deliver the final product.

One controversial provision would exempt HHS from releasing information obtained through BARDA that could "reveal vulnerabilities" to bioweapons. Groups such as the Washington, D.C.–based Center for Arms Control and Non-Proliferation say that's too vague and could keep important research secret.

The Senate has begun considering a similar bill, S. 2564, sponsored by Richard Burr (R–NC). Supporters are hopeful that it will pass during the Senate's lame-duck session after the November elections. Differences in the two bills—for instance, the Senate version includes antitrust provisions—would have to be reconciled in a conference.

Meanwhile, HHS promises to spell out details of the existing BioShield program, such as exactly which biological agents HHS is interested in and whether it wants oral or injected vaccines, in an implementation plan in early 2007. **–JOCELYN KAISER**

CREDIT: GUILLAUME HORCAJUELO/APERCU



John Niederhuber applies a hands-on approach to running NIH's largest institute—and retains ties to the lab and the cancer clinic

A Physician-Scientist Takes the Helm of NCI

IN THE SUMMER OF 2005, 67-YEAR-OLD cancer surgeon John Niederhuber was ready for a new chapter in a career spent hopscotching across the country in academic medicine. His wife had died of breast cancer a few years earlier, and his son would soon head off to college. So when Andrew von Eschenbach, director of the National Cancer Institute (NCI), asked him to join his staff as a deputy director, Niederhuber left his job as a surgery and oncology professor at the University of Wisconsin, sold his house in Madison, and headed to Washington, D.C.

The idea was to get back into the lab as well as to coordinate NCI's translational and clinical programs. But that plan was scrapped after President George W. Bush asked von Eschenbach to fill a sudden vacancy at the top of the Food and Drug Administration, then nominated him as FDA commissioner. In August, the White House quietly announced that Niederhuber, then acting NCI chief, would become the institute's 13th director. "Why I'm here, I don't know," he tells *Science* in an interview in his office suite on the National Institutes of Health (NIH) campus in Bethesda, Maryland. "One of those fates in life has put me here. I'll do my damnedest to do the best job I can … to serve the American people and the research community."

Longtime colleagues say such modesty and dedication are hallmarks of the tall, soft-spoken, Ohio-born biomedical research administrator, who by turns has headed a large cancer center, been an outstanding oncologic surgeon, and run an immunology lab. "John understands the politics of cancer, the patient care aspect of cancer, the basic science. He has a unique combination of talents," says Allen Lichter, who recently stepped down as dean of the University of Michigan Medical School to become chief executive officer of the American Society of Clinical Oncology in Alexandria, Virginia. "I don't think you could find someone who was better prepared."

Niederhuber inherits NIH's largest institute, but one whose $4.8 billion budget has been flat 2 years running and is likely to remain so in 2007. One of his first moves has been to scrap the management model favored by von Eschenbach, who considered himself a big-picture CEO representing NCI in the cancer community while entrusting daily management to top-level deputies. Instead, Niederhuber interacts daily with the institute's seven division directors. Together, the team is rating existing programs and looking for cuts throughout the institute, including the $240 million in administrative funds that now go to the director's office. Niederhuber also has declined to embrace one of his predecessor's most visible—and controversial—goals: to eliminate suffering and death from cancer by 2015. Instead, Niederhuber says he prefers to "lessen the burden of cancer"—without setting a target date.

Some of his moves seem designed to send a message to the cancer community. His decision to set up his own small lab with a staff scientist and two postdocs to study the role of stem cells in tumor growth is seen by NCI intramural scientists as a signal that he's one of them. And a 3-year, $9 million pilot project to bring cutting-edge cancer treatments to community hospitals reflects his strong belief that giving the average patient access to new science will lower mortality rates.

One von Eschenbach priority that he supports is a collection of big new technology programs such as nanotechnology and bioinformatics. Some researchers say the programs are taking money from the bread-and-butter R01 grants to individual investigators and would like to see an outside

CREDIT: MARTY KATZ

Back to basics. Niederhuber reviews results from his small NCI research lab.

review of their value. Instead, Niederhuber has resisted calls to shift funds wholesale to the R01 pool, citing the need to stay in line with NIH-wide policy to keep grant numbers steady rather than have pay lines that vary widely among institutes.

Friends and colleagues are pleased with his appointment. "I think he's going to be a terrific director at a really difficult time. I hope the outside community appreciates how talented he is," says Bruce Chabner, clinical director at the Massachusetts General Hospital Cancer Center and a former NCI division director on whose board Niederhuber chaired. Robert Wiltrout, head of the institute's $367 million intramural Center for Cancer Research, says, "I think we're very lucky to have found John. He focuses attention on those around him, which builds morale." And John Mendelsohn, president of the University of Texas M. D. Anderson Cancer Center in Houston, calls Niederhuber "a calming influence. [He is] reaching out and bringing into his deliberations advice from just about all the stakeholders I can think of. We're all ready to roll up our sleeves and work with him."

Traveling man

Niederhuber's roots as a physician-scientist go back to Bethany College, a small liberal arts school in West Virginia, where as an undergraduate he had his own chemistry lab. After picking up a medical degree from Ohio State University, he considered a Ph.D. program, partly to avoid being drafted for the war in Vietnam. When the Army eliminated that exemption, he instead joined as an officer and wound up at the U.S. Army Biological Laboratory at Fort Detrick, Maryland, for 2 years. After a postdoc in immunology at the Karolinska Institute in Sweden, he completed a residency at the University of Michigan and then joined its faculty, garnering an NIH career-development award and then an R01 in immunology while also pioneering pumps that can be implanted under the skin to deliver drugs directly to the liver. His 14 years at Michigan, he says, "gave me an appreciation for why both basic scientists and clinicians get up in the morning."

Lured to Johns Hopkins University in Baltimore, Maryland, in 1987, he conducted both clinical and basic research, studying the role in cell growth of tyrosine kinases—including *blk*, an oncogene his lab discovered. But after 4 years, he headed west to join Stanford University School of Medicine Dean David Korn in building an academic

CREDIT: MARTY KATZ

Budgets, Patients, Managing Conflicts

In a 21 September interview with *Science*, John Niederhuber talked about major issues facing the National Cancer Institute (NCI).

ON MANAGEMENT:

My style is more to work directly with the division leadership. We've moved our executive committee meetings to almost once a week from twice a month before. … We have great leadership across the divisions at this time. I'm not going to have that layer of what's called the senior management team. …

Intramural and extramural [division directors are rating each other's programs by anonymous ballot]. Yesterday, we decided there are certain concepts we wouldn't take forward to the BSA [Board of Scientific Advisors] … [because] we couldn't really give them a high enough priority. We're also trying to say, is there something that's outlived its usefulness. This may also be an extramural activity which … we can begin to plan how to phase out. … I have been tremendously impressed with how well the division leadership have been willing to work together in a very collegial way to make collective decisions, hard decisions.

ON THE R01 PAY LINE:

This is a very stressful time, budgetwise. I know full well, because I've lived through those periods of single-digit pay lines [the funding cutoff for grant applications, as ranked by peer review] in my own lab. … A big part of my responsibility is to help the leadership of NCI manage that [congressional] appropriation as effectively as we can.

[But regarding efforts to raise the pay line], a lot of that decision is made at the NIH level. We don't want to have one institute having a 5% success rate and another one 20%. At NIH, we want to try to minimize that variability as much as we can. And so we try to make corporate-level decisions and targets. Some of those are to try to maintain the same number of competing awards that we had in the previous year, just as an example. … We would probably … try to keep the size of those grants about the same.

ON CONFLICT-OF-INTEREST RULES:

I feel sad in many ways about Tom [Walsh, an NCI expert on fungal diseases who violated rules on reporting outside income] and some of the other individuals. I think these are very well-meaning people. Certainly, Tom couldn't be a harder worker in terms of delivering outstanding patient care. He's as committed as anyone to NCI, NIH. …

But you know, there are rules and regulations, and none of us, no matter how good we are, are above those rules and regulations. … It's always easy to look for excuses, and maybe one of the excuses is the environment perhaps was not paying as close attention as it should have. There is sometimes a little bit of this absent-minded professor issue. … Even in academia and universities, many of us have struggled with this.

ON PRIORITIES:

I have been talking a lot about a continuum in this process of research and science that leads to impacting on patient outcome. … What I call a chemical space, … a biologic space, and a translational space. One of the things we're going to work hard on is the integration of those spaces. I see that integration process driven by our investment and work in technology development. …

The community [cancer centers] program is important as an underpinning of that because one of the greatest challenges for us in the future will be getting science to patients where they live. … If we work in this continuum, we can do a lot to drive down the cost of drug discovery. … I think you're going to see that dramatically change with the technology developments and investments that we'll make.

ON HIS LAB:

It's exciting for me to get the lab going again. … Monday morning, we have a very formal almost 3 hours, then I'll pop over at the end of the day every now and then. … The team here knows that that [scheduled lab] time can't be violated, even by the president. **–J.K.**

surgery department. Dealing with sensitive tasks such as merging Stanford's faculty practice plan with its hospital took a toll, however, and after Korn left in 1995, Niederhuber headed to the University of Wisconsin to "get back into the cancer business."

At Wisconsin, he oversaw a challenging merger of basic and clinical cancer centers while coping with a recurrence of his wife's breast cancer; she died in 2001. After clashing with the medical school dean over raising money for the cancer center, Niederhuber stepped down as director by "mutual decision" and let his R01 lapse. Then last year, he answered the call from von Eschenbach, with whom he was already working as chair of the presidentially appointed National Cancer Advisory Board.

His ascension to the directorship wasn't a shoo-in, however. Last spring, 62 prominent cancer researchers urged the White House to conduct a broad search after von Eschenbach was tapped for the FDA post (*Science*, 21 April, p. 357). Several candidates were interviewed, although no official search committee was formed. One of them, Joseph Pagano, the 74-year-old former director of the Wake Forest Lineberger Cancer Center in North Carolina, said he told his interviewers that the president should appoint someone younger. Pagano likes Niederhuber's "excellent scientific intellect" and grounding in basic science, something that he says von Eschenbach lacked. "I think he's a very good man."

Niederhuber, for his part, says he feels he's been warmly welcomed by both the basic and clinical cancer communities. "I haven't sensed that I have to prove anything," he says.

–JOCELYN KAISER

NEURODEGENERATIVE DISEASES

Picking Apart the Causes of Mysterious Dementias

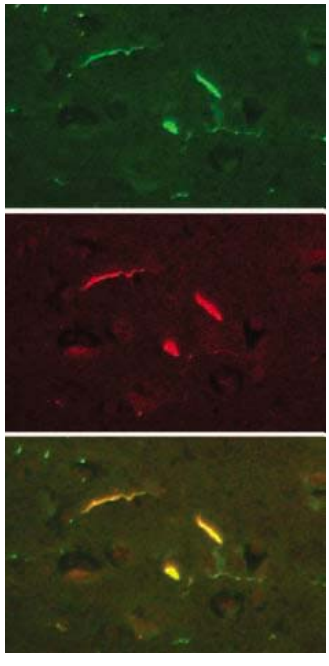
Researchers are identifying the genetic and biochemical underpinnings of frontotemporal lobar dementias, incapacitating and ultimately fatal conditions

As we grow older, we face a host of diseases that can rob us of our mental vigor. Alzheimer's is the best known of these neurodegenerative diseases, but others can be just as devastating. Take the group of diseases collectively known as the frontotemporal lobar dementias (FTLDs).

As the name suggests, these conditions stem from degeneration in the frontal and temporal lobes of the brain, areas that control behavior, emotions, and language. The symptoms, which usually develop when people are in their 50s or early 60s, include language difficulties and inappropriate behavior. Patients with early-stage FTLD typically can't control their impulses and may shoplift, overeat, and show excessive interest in sex. A decline in personal hygiene is also a frequent symptom.

Once thought to be rare, FTLDs are gaining new respect as a significant cause of dementia. Good estimates of their prevalence are hard to come by. But Andrew Kertesz, an FTLD expert at the University of Western Ontario in London, Canada, says that about 12% of patients treated at dementia clinics have FTLDs. He notes, however, that this could be an underestimate because the conditions may be misdiagnosed as psychiatric disorders, at least in their early stages, or as Alzheimer's disease.

Several recent reports, including one on page 130 of this issue, are beginning to identify the genetic and biochemical abnormalities underlying FTLDs. Neurobiologist Michael Hutton of the Mayo Clinic College of Medicine in Jacksonville, Florida, is impressed with the speed of the discoveries. "It's like compressing 10 years of Alzheimer's disease research into 6 months," he says.



Overlap. In FTLD cells, ubiquitin (green) and TDP-43 (red) colocalize (yellow).

CREDIT: M. NEUMANN *ET AL.*, *SCIENCE*

In addition, some of the work has solidified a connection between FTLDs and another neurodegenerative disorder, amyotrophic lateral sclerosis (ALS), better known as Lou Gehrig's disease. It suggests that the underlying pathology may be similar in both sets of diseases. If so, therapies directed at one might also work on the others. Because neither FTLDs nor ALS can be treated now, both are ultimately fatal.

Pick's disease

Alzheimer's disease deserves its widespread notoriety, but the lesser known FTLDs have a longer history. The Czech neurologist Albert Pick described the first case in 1892, 16 years before Alois Alzheimer discovered the dementia that bears his name.

For decades, cases of dementia caused by frontotemporal lobe damage were known simply as Pick's disease, but neurologists gradually learned that the condition is very heterogeneous. "Even in the same family, siblings have different symptoms," says neurobiologist Virginia Lee of the University of Pennsylvania School of Medicine in Philadelphia.

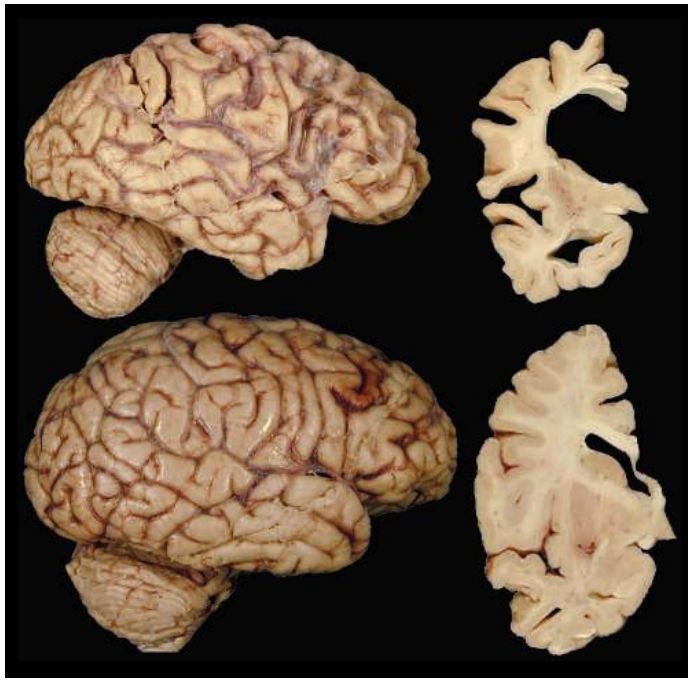
The variation in FTLD symptoms may reflect differences in brain pathology that neurobiologists are finding. As with Alzheimer's disease, ALS, and many other neurodegenerative diseases, FTLDs are characterized by the presence of abnormal protein deposits in affected neurons. Beginning about 15 years ago, researchers showed that in about half the people with FTLD, the deposits contain the protein tau, which has also been implicated in Alzheimer's pathology. Eight years ago, several teams provided genetic evidence that mutations in the *tau* gene itself can produce this particular form of FTLD, which includes the classic Pick's disease.

The identities of the proteins in the inclusions of the remaining half of FTLD cases have been harder to pin down. Researchers did find, however, that the inclusions in these tau-negative cases are tagged with ubiquitin, a small protein that the cell uses to mark proteins for destruction.

Even within this subclass of FTLDs, researchers are now finding pathological variations. Using antibodies that recognize ubiquitin, Lee, John Trojanowski, also at the University of Pennsylvania School of Medicine, and their colleagues examined the

structure and distribution patterns of the inclusions in brains taken from such FTLD patients at autopsy. They found three distinct patterns, indicating that the cases could be subdivided into subtypes. (The findings appear in this week's *American Journal of Pathology*.)

Now, the Lee-Trojanowski team has used two of the same antibodies to identify the first protein, beyond ubiquitin, in the tau-free inclusions. As described on page 130, the researchers found a protein called TDP-43 that, Lee says, "was present in all the brains of patients with all subtypes" of FTLD with ubiquitin-positive, tau-free inclusions. What's more, Lee and her colleagues detected TDP-43 in the ubiquitinated inclusions within the neurons of ALS patients.



Brain damage. Compared to a normal brain (*bottom images*), a brain from someone with FTLD shows shrinkage of the frontal and temporal lobes (*upper left*) and an enlarged ventricle (*upper right*).

CREDIT: D. W. DICKSON/NEUROPATHOLOGY LABORATORY, MAYO CLINIC JACKSONVILLE

FTLDs and ALS had already been linked clinically. ALS is best known as a destroyer of the body's motoneurons, ultimately leading to death as the muscles lose all ability to contract. But many people with ALS also develop dementia. Conversely, people with FTLDs frequently develop motoneuron disease similar to that of ALS.

The identification of TDP-43 in both FTLD and ALS inclusions now provides a biochemical link between the two conditions. They "could be different parts of a spectrum of diseases," says Ian Mackenzie of the University of British Columbia in Vancouver, Canada, who is a co-author of the Lee paper.

Neurobiologists are hopeful that the TDP-43 finding can help unravel the underlying causes of both neurodegenerative diseases. Robert Brown, an ALS expert at Harvard's Massachusetts General Hospital in Boston, points out that the appearance of threads of ubiquitinated protein in neurons is "one of the earliest signs of pathology in ALS, and we had no hint at all of what the abnormal protein is." The Lee team's observation, he adds, "means that we have some clue of what distinguishes ALS neurons as being abnormal."

At present, however, TDP-43's function in neurons and elsewhere is pretty much a mystery; PubMed contains only 12 references to the protein. There are some indications that it functions in the nucleus, perhaps as a regulator of gene expression or as a structural protein or both. And in the current work, the Lee team found that levels of nuclear TDP-43 are much lower in FTLD neurons than in normal ones; the protein is instead concentrated in inclusions, which are located in the cytoplasm. It remains unclear whether the FTLD neurons die because the inclusions are somehow toxic or because TDP-43's nuclear function has been lost.

Help from genetics

Both FTLD and ALS are known to run in families, and researchers are making progress in identifying the genes at fault in these hereditary cases. One such discovery, from John Collinge and colleagues at University College London in the United Kingdom, buttresses the link between FTLD and ALS. Last year, the team reported that mutations in a gene called *CHMP2B* cause FTLD in a Danish family. In work reported this summer in *Neurology*, the researchers have now linked *CHMP2B* mutations to two cases of ALS. "It supports the view that [FTLD and ALS] may have common etiologies," Collinge says.

So far, *CHMP2B* mutations appear to be a rare cause of FTLD, but the past 6 months have seen the identification of two additional FTLD genes—perhaps another indication of the dementia's heterogeneity. In the June issue of the *Journal of Neuropathology & Experimental Neurology*, Virginia Kimonis of Children's Hospital Boston and colleagues link a subset of cases to mutations in the gene for the valosin-containing protein (VCP). And in work published online by *Nature* in July, two independent teams, one led by Mackenzie and Hutton and the other by Christine van Broeckhoven of the University of Antwerp, Belgium, connect a different subset to mutations in the gene for a protein called progranulin. Since then, at least three more groups have weighed in with progranulin mutations in FTLD cases.

Neither VCP nor progranulin appears to be located in FTLD inclusions. In the case of progranulin, there's a good explanation, says Mackenzie. The progranulin gene mutations identified result in loss of protein production.

At present, the supposition is that loss or alterations in protein function caused by the various mutated FTLD genes trigger changes in neurons that somehow lead to formation of the abnormal inclusions containing ubiquitinated TDP-43. Although no one yet knows how that happens, the nature of the proteins normally encoded by the mutant genes points to the possibility that their malfunction leads to an abnormal buildup of proteins that have to be removed—a process for which ubiquitin addition is the first step.

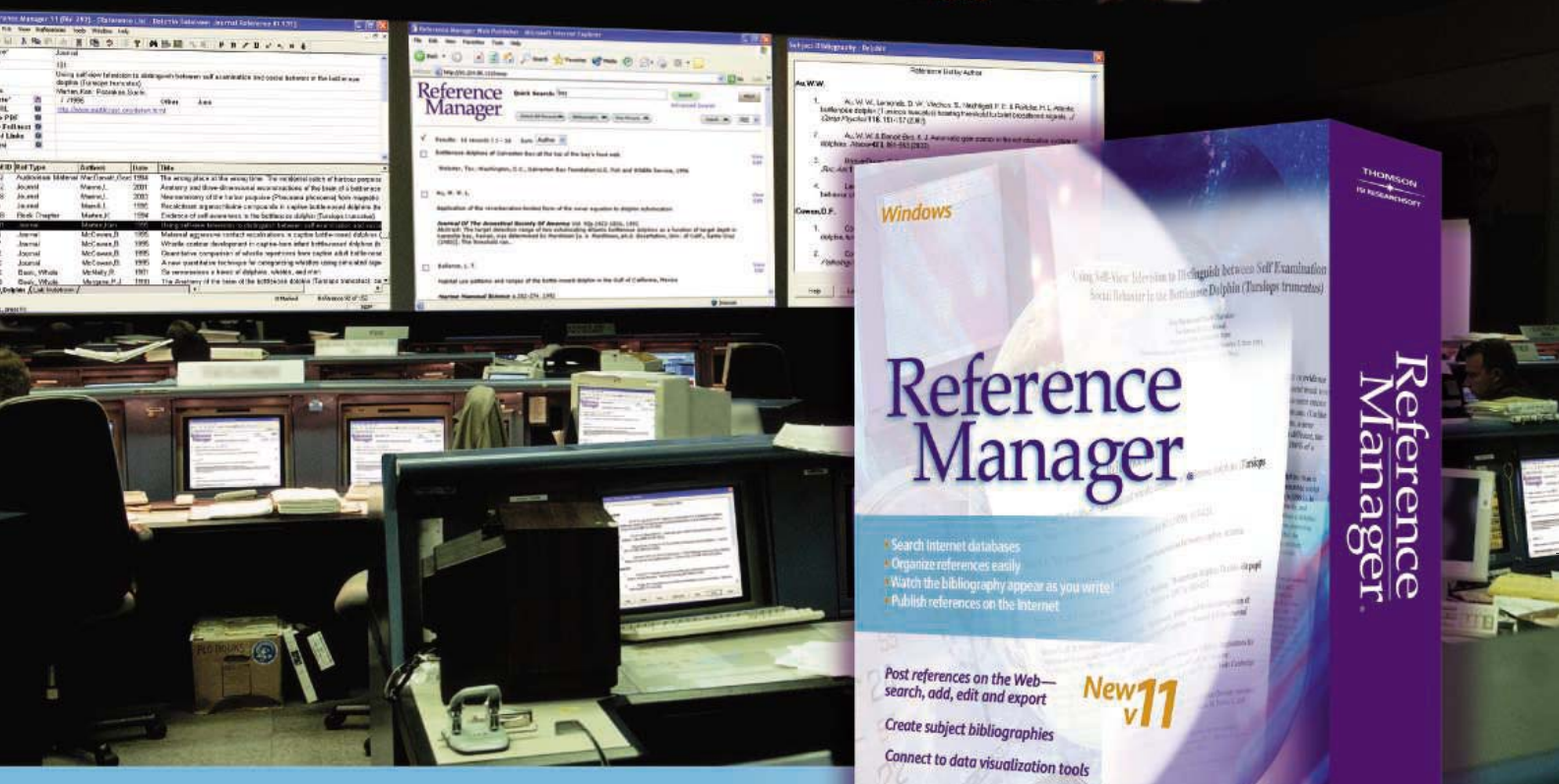
VCP and CHMP2B, for example, are directly involved in protein folding and trafficking in the cell. As for progranulin, it is a growth factor that might be needed for neuronal maintenance, although it is also a promoter of blood vessel formation. Failure to maintain a normal blood supply to cells can produce oxidative stress, a known inducer of the so-called unfolded protein response, which has been linked to neurodegeneration (*Science*, 15 September, p. 1564).

A similar mechanism may also come into play in ALS. Brown and Orla Hardiman of the Royal College of Surgeons in Ireland in Dublin this spring identified mutations in the *angiogenin* (*ANG*) gene as a cause of ALS in Irish and Scottish populations. *ANG*, like progranulin, encourages blood vessel growth. And previously identified ALS-causing mutations in the *SOD1* gene may lead to oxidative stress, too.

Neurobiologists clearly have a lot to do before they can explain the causes of tau-negative FTLDs and ALS, but the flurry of recent advances has made them hopeful. "We've got a genetic cause, and we know what protein is accumulating," Hutton says. "Now we have to connect the dots." Pick would be proud.

–JEAN MARX

BIBLIOGRAPHY CENTRAL



Your High-Tech Command and Control Center for References.

Introducing Reference Manager 11—a powerful upgrade to the bibliographic software that streamlines research, writing and publishing.

Reference Manager has served corporate, government and academic researchers worldwide for over 20 years. And now version 11 delivers new ways to share and view your reference collections: Post your databases to the Web. Collaborate with colleagues over a network. Link to full text pdf files.

These are just some of the powerful features that await you. Reference Manager is your command and control center for all things reference related.

What's new in v11:

- Publish Reference Manager databases to the Web or intranet
- Create subject bibliographies instantly
- Access new and updated content files at www.refman.com
- Share traveling libraries with colleagues
- Connect to data visualization tools

Put innovation into action. Order or upgrade today.
Available for Windows in a single-user and network edition.
Phone: 800-722-1227 • 760-438-5526 • info@isiresearchsoft.com

Download a Free Demo Today
www.refman.com

THOMSON
ISI RESEARCHSOFT™

SCIENCE POLICY

Priorities Needed for Nano-Risk Research and Development

Nanotechnology observers are split over the best way to ensure that the up-and-coming industry remains safe for both people and the environment

A broad array of nanotechnology experts agree that the United States needs to spend more money on understanding potential health and environmental dangers of exposure to materials engineered on the scale of a few clumps of atoms. But just how that research should be prioritized and organized is a topic of increasingly fierce debate.

The potential adverse impacts of nanotechnology sprang to the fore again at a sometimes-contentious hearing of the U.S. House Science Committee on 21 September. At the hearing, leaders of the Nanotechnology Environmental and Health Implications (NEHI) working group—an interagency panel that coordinates federal funding on health and environmental risks of nanotechnology—released a long-overdue report outlining research needed to buttress regulation of products in the field. But critics both inside and outside Congress blasted the report as a jumbled wish list. "The government needs to establish a clear, prioritized research agenda and fund it adequately. We still haven't done that, and time is a-wasting," says committee chair Sherwood Boehlert (R–NY).

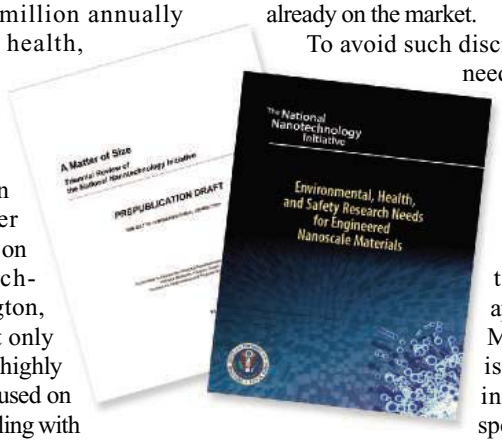
There is certainly plenty riding on how nanotechnology is regulated. More than 200 nanotechnology products are already on the market, including sunscreens and cosmetics, lightweight bicycle frames, and car wax, and they accounted for more than $32 billion in sales last year. A recent market survey by Lux Research, a nanotechnology research and advisory firm in New York City, predicts that by 2014, a whopping $2.6 trillion worth of manufactured goods will incorporate nanotechnology. "The nanotechnology industry, which has enormous economic potential, will be stymied if the risks of nanotechnology are not clearly addressed and understood," Boehlert says.

That is already happening, says Lux Research Vice President Matthew Nordan. At the hearing, Nordan said that Lux has learned through its private consulting work that some Fortune 500 companies are already backing out of nanotechnology research because of real and perceived risks of nanomaterials and uncertainties over how they would be regulated. Venture-capital funders and insurers have also pulled their services for some clients for the same reason, Nordan says, although he didn't offer specifics.

To stem this tide, Nordan and other experts argue that nanotoxicology research funding should be increased dramatically. According to figures from the U.S. National Nanotechnology Initiative, federal agencies currently spend a combined $38.5 million annually on environmental, health, and safety research on nanotechnology. Last year, however, researchers at the Woodrow Wilson International Center for Scholars' Project on Emerging Nanotechnologies in Washington, D.C., concluded that only $11 million went to "highly relevant" research focused on understanding and dealing with the risks of nanomaterials (see table). At a congressional hearing last year, nongovernmental experts called for raising funds for such studies to between $50 million and $100 million a year (*Science*, 9 December 2005, p. 1609). Both the NEHI report and another report released on 25 September by the National Research Council echoed calls for expanding research in the field.

But there is far less agreement on how that money should be spent and coordinated. "Nanotech [environmental health and safety] research in government agencies, academic institutions, and industry is being performed in an ad hoc fashion according to individual priorities," Nordan says. That scattershot approach has left broad gaps between what the agencies are pursuing and what is needed to tune regulations to products already on the market, argues Andrew Maynard, chief scientist of the Wilson Center's Project on Emerging Nanotechnologies. For example, Maynard says, carbon-based nanomaterials are incorporated into only about one-third of nanotech products. Yet the vast majority of nanotoxicology studies focus on those materials, while ignoring broad classes of other materials already on the market.

To avoid such discrepancies, agencies need a more centralized top-down research approach, Nordan and Maynard argue. "What is missing is not [an] ingredients list but a specific game plan for accomplishing this research," Nordan says.



Chorus line. Two new reports call for increased funding for nano-safety studies.

But NEHI leaders and other agency brass say a federal priorities list is coming and maintain that the current coordination scheme is the best way to implement it. "The coordination that is taking place is working," argues Celia Merzbacher, a member of the President's Office of Science and Technology Policy as well as the co-chair of the Nanoscale Science, Engineering, and Technology (NSET) Subcommittee of the National Science and Technology Council. Furthermore, she adds, "our approach achieves the buy-in of the agencies." In addition to the NEHI working group, she notes, there is already a full-time National Nanotechnology Coordination Office (NNCO) within NSET. "We don't need another coordination office," she says. But Nordan counters that coordinating bodies such as NEHI and NNCO have no authority to mandate priorities and can't allocate funding.

With the strongest calls for reform still coming from outside government, a shakeup of nanotechnology research looks unlikely anytime soon. At the hearing, Boehlert did argue that "current coordinating mechanisms clearly are inadequate." But Boehlert is retiring at the end of his current term on 31 December. And it remains to be seen whether other congressional science leaders will emerge to pick up the baton.

–ROBERT F. SERVICE

U.S. Federal Nanotech Risk R&D
(Fiscal Year 2006; in $ millions)

Agency	NNI (est.)	"Highly Relevant" R&D (Project on Emerging Nanotech)
NSF	24.0	2.5
DOD	1.0	1.1
DOE	0.5	0.0
NIH	3.0	3.0
NIOSH	3.07	1.9
DOC	0.9	0.0
USDA	0.5	0.0
EPA	4.0	2.3
DOJ	1.5	0.0
Total	**$38.47**	**$10.8**

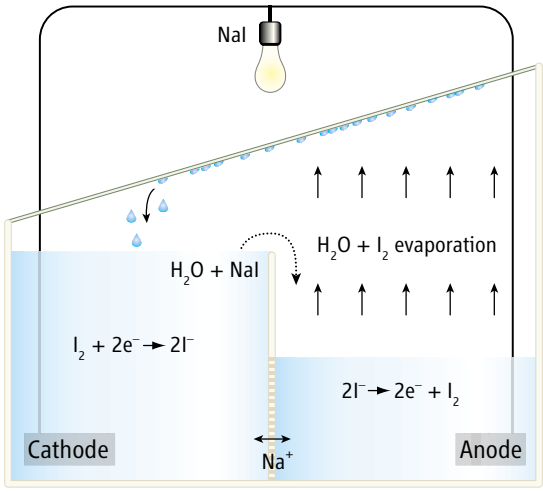
Stopgap. Little nano-risk research is conducted by agencies that oversee health and environmental regulations.

SOURCE (BOTTOM): ADAPTED FROM A TABLE BY THE WOODROW WILSON INTERNATIONAL CENTER FOR SCHOLARS' PROJECT ON EMERGING NANOTECHNOLOGIES

Power to the (Poor) People

The estimated 25% of the world's people without electricity face a nearly inescapable cycle of poverty, poor health, and lack of education. Unfortunately, large-scale centralized power plants are expensive, and efforts to tap widespread natural low-temperature heat sources such as geysers, hot springs, and peat bogs to run generators have largely proved ineffective. But new work offers a different way to squeeze at least a modest amount of electricity from those hot spots.

At the meeting, Roman Boulatov, a chemist at the University of Illinois, Urbana-Champaign (UIUC), reported a new device akin to a fuel cell that uses reactions of charged compounds to create electricity. But whereas a fuel cell mines energy from chemical fuel such as hydrogen gas, the new device—called a thermally regenerative solution concentration cell (TRSCC)—is recharged using freely available heat. The TRSCC is only 1% efficient at turning heat to electric power, so it won't be charging any major cities. But because it can be made from cheap materials, it could be a godsend to those who have few other options. "Clearly, this is an interesting idea," says Paul Kenis, a fuel cell expert at UIUC, who is unaffiliated with Boulatov's work. "It may be a solution for developing countries or remote areas of the world that lack access to the power grid."



Energy solution. Free heat can regenerate iodide (I^-) ions, which can be stripped of their electrons to create a current.

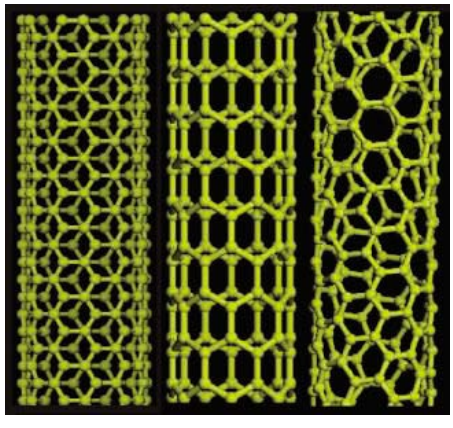
Boulatov made the device along with Michal Lahav when both were postdoctoral assistants in the group of Harvard University chemist George Whitesides. Like a fuel cell, it consists of two compartments—each with an electrode submerged in water—separated by a semipermeable membrane. In Boulatov's TRSCC, the side containing the positively charged anode is spiked with a high concentration of sodium iodide (NaI), which in solution dissolves into a mixture of positively charged sodium ions (Na^+) and negatively charge iodide (I^-). The other chamber contains the same salt at thousands of times lower concentration. Both chambers also contain a small amount of neutrally charged iodine (I_2). The large I^- concentration difference drives the solutions to equilibrate. But because the negatively charged I^- ions can't move through the membrane, they must carry out their task at the electrodes. In the chamber with the high I^- concentration, the positively charged anode swipes an electron from two iodides, generating an I_2 molecule. The electrons then move through a wire, where they can be used to do work en route to the other chamber. When they reach the negatively charged cathode, they combine with a molecule of I_2, which splits into two I^- ions. Meanwhile, positively charged sodium ions move through the membrane to maintain the electrical neutrality of each solution.

On its own, this reaction would quickly balance out the amount of iodide and iodine in each chamber, and the cell would go dead. To keep it running, the researchers heat the high-I^- chamber. Water and I_2 evaporate from the solution and then, by design, condense in the second chamber. As the level of solution in the cathode chamber rises, it flows over a barrier between the two chambers, carrying with it regenerated I^- as well as Na^+ to replenish NaI on that side. The upshot is that the added heat maintains a difference in concentration of I^- between the chambers, thereby keeping the reaction going. In initial tests, Boulatov says his TRSCC has kept running for 350 hours.

If several dozen such chambers were set up side by side, they could provide enough power at a high enough voltage to run simple machines such as telephones, water purifiers, and refrigerators, Boulatov says. That could begin to help large numbers of people climb out of hopeless poverty.

Cut-and-Copy Approach Clones Nanotubes

When Rice University chemist Richard Smalley died of cancer last October, nanotechnology lost one of its most adept practitioners and inspiring visionaries. High on Smalley's wish list for the field was a way to produce just one type of carbon nanotube at a time.



Variety pack. A new technique amplifies a single electronic flavor of nanotubes.

Nanotubes are made from sheets of carbon atoms rolled up into tubes. But just how those sheets roll up makes some tubes behave like metals and others like semiconductors. That difference is critical for researchers hoping to use nanotubes to make ultrasmall transistors and sensors and long wires and cables. Conventional schemes for making the purest form of nanotubes, called single-walled nanotubes (SWNTs), create blends of all the possible varieties. So Smalley emphasized the need to isolate just one electronic flavor.

At a 4-day symposium dedicated to Smalley, who shared the 1996 Nobel Prize in chemistry for his discovery of fullerenes, a collaboration of four research groups at Rice in Houston, Texas, reported discovering a way to clone just a single electronic type of

CREDITS (TOP TO BOTTOM): ILLUSTRATION: P. HUEY/SCIENCE; JAIDEEP LAMBA/NANOTECH INSTITUTE, UNIVERSITY OF TEXAS, DALLAS

nanotube, leaving the others out. If the new scheme can be scaled up to produce industrial quantities, it could open up a wide variety of electronics applications of carbon nanotubes. "It's very promising," says Jie Liu, a chemist at Duke University in Durham, North Carolina. However, he cautions that the Rice team still has a way to go to prove that the process does indeed produce just a single type of tube.

The teams took their cue from a technique that uses tiny nano-sized catalyst particles to seed the growth of tubes. About 5% of catalyst particles in a reactor give rise to new tubes, from hundreds of nanometers to micrometers long, says Andrew Barron, a Rice University chemist, whose group, along with those of James Tour, Ed Billups, and Smalley's own former group, carried out the new work. Barron and his colleagues set out to improve the "seeding" step by making better use of the small percentage of tubes that do grow well.

They started by isolating bunches of SWNTs with different electronic properties. They then cut the tubes into tiny straws, most of which were about 40 nanometers long, and reacted them with a compound that left carboxylic acid groups attached to the ends of the tubes. They added the tubes to a vacuum chamber with catalytic iron oxide nanoparticles, which readily adhere to the carboxylic acids. Once the catalysts were attached, they removed the intervening carboxylic acids by heating the tubes, either alone or with hydrogen. Finally, they added a carbon gas feedstock, such as carbon monoxide, methane, or acetylene, at 900°C. At that temperature, the feedstock gases break apart, and carbon atoms insert themselves between the catalyst particles and the starting portion of the tube, extending the tubes to long lengths.

Before-and-after images of the tubes using Raman fluorescence, a technique sensitive to the arrangement of carbon atoms in the tubes, didn't detect any change in the type of tubes in the mix or in their relative abundances, Barron says. Still, the technique works only with semiconducting tubes, not the metallic variety, so the Rice team is working to verify that all the tubes are indeed clones.

If they are, and if the technique can be scaled up, that would be a boon to researchers looking to weave millions of SWNTs together to make long, thick power cables with minute electrical resistance. Well-sorted nanotubes could also make possible a new generation of sensors and tiny nanoelectronic devices. Those would go a long way toward fulfilling Smalley's vision.

–ROBERT F. SERVICE

CREDITS (TOP TO BOTTOM): SHAOLI FANG; YUHUANG WANG/NORTHWESTERN UNIVERSITY

Snapshots From the Meeting >>

First nanotube textile. Not a bad way to get your career in science off the ground. Plano, Texas, high school student Diane Chen is shown here weaving the first ever carbon nanotube–based textile. For a summer research project, Chen used a conventional wooden loom strung with polyester yarn and wove in 10-ply yarn made from multiwalled carbon nanotubes synthesized in the lab of Raymond Baughman, a materials scientist at the University of Texas, Dallas. Although Baughman said his team has yet to measure the properties of the fingernail-sized swatch of fabric, nanotube textiles are expected to be extremely strong and flexible and have the potential to be used for energy storage, sensors, and even artificial muscles.

Evolving better hydrogenases. Bacteria figured out the route to clean, green energy more than a billion years ago. *Escherichia coli* and many other microbes harbor enzymes called hydrogenases that use iron and nickel to split water to generate hydrogen gas, from which they then extract energy. Researchers would love to be able to employ such catalysts on a massive scale to generate fuel to power the hydrogen economy in the future. One big challenge is that hydrogenases don't work in the presence of oxygen, a trait that makes them hard to exploit industrially.

Researchers at Stanford University in California, however, have begun tackling that problem. They've devised a "cell-free" way to synthesize large numbers of mutant proteins—in this case hydrogenases—and rapidly screen them for catalytic activity. So far, they've turned out some 40,000 mutant hydrogenases, a small percentage of which are able to tolerate the presence of oxygen. However, their catalytic rate is still low. But if scientists find one that's both oxygen-tolerant and highly active, it could help solve one of the biggest stumbling blocks on the road to abundant carbon-free energy.

Scribble, scribble. Ever since researchers at IBM first used a scanning probe microscope to write their company logo in xenon atoms back in 1990, critics have complained that the probe's single tip made the technique far too slow. Not any more. Chad Mirkin and colleagues at Northwestern University in Evanston, Illinois, and NanoInk Inc. reported at the meeting that they've created an array of 55,000 microscopic tips that can spot down different chemicals simultaneously, covering a full square centimeter at time. **–R.F.S.**

EDITED BY YUDHIJIT BHATTACHARJEE

Two Cultures

BEAUTIFUL BRAINS. To neuroscience researchers, the human brain is a complex organ riddled with mystery. But to developmental psychologist Marjorie Taylor and psychiatrist Karen Norberg, it's also an inspiration for unique quilts, knitting, and other work that they showcase in an online museum of "scientifically accurate fabric brain art."

The two were drawn to the niche independently. Taylor (right), a professor at the University of Oregon, Eugene, had been making quilts on the side for years before she turned her needle to neuroscience. Struck by the cover images of journals like *Cerebral Cortex*, she began reproducing them in fabric, creating pieces that—for example—show positron emission tomography scans of the brain's response to hearing or seeing words.

Karen Norberg (left), who works at the National Bureau of Economic Research in Cambridge, Massachusetts, says she began knitting a brain (above) to kill time when she was undergoing clinical training in child psychiatry. The product now resides at the Boston Museum of Science.

"Building a brain with yarn and knitting needles turns out to follow many of the same pathways as actual brain development," says Norberg. Her and Taylor's work can be seen at harbaugh.uoregon.edu/Brain/index.htm.

CREDITS (TOP TO BOTTOM): TOP AND TOP LEFT: KAREN NORBERG; TOP RIGHT: JIM BARLOW/UNIVERSITY OF OREGON; STANFORD UNIVERSITY NEWS SERVICE; © 2005, *THE WASHINGTON POST*. PHOTO BY LUCIAN PERKINS. REPRINTED WITH PERMISSION

AWARDS

DIGITAL PATHWAY. Stanford University electrical engineer John Cioffi, who helped bring Internet connections to millions of people through the digital subscriber line (DSL), has won the 2006 Marconi Prize.

In 1993, Cioffi (left) and his team built a high-speed modem for communicating data through phone lines that eventually became the standard for broadband Internet connection. He later created Dynamic Management Spectrum, which offers more bandwidth than DSL. In addition to holding more than 70 patents, Cioffi also owns several companies. He plans to give his $100,000 prize, from the Marconi Society at Columbia University, to his research group.

STICKING WITH CDC. Efforts to prepare the U.S. and the world for a flu pandemic have won Nancy Cox, a top flu expert at the Centers for Disease Control and Prevention (CDC) in Atlanta, Georgia, the title of "Federal Employee of the Year." The award is issued every year by the Partnership for Public Service, a nonprofit.

Cox, 58, joined CDC in 1975 and has been at the helm of its influenza program since 1992. Although CDC has suffered an exodus of veteran scientists recently in the wake of a reorganization, Cox says it's a good place to work: "As a child, I never dreamed I'd have the opportunities I've had while working at CDC."

Got a tip for this page? E-mail people@aaas.org

Three Q's >>

Susan Wood made a stir a year ago when she quit the U.S. Food and Drug Administration (FDA) in protest after the agency refused to make emergency contraception available over the counter. (FDA later reversed its decision.) Now, Wood is back in the news as a co-founder of Scientists and Engineers for America (SEA), a political action committee backing pro-science congressional candidates in next month's elections.

Q: What does SEA hope to accomplish?

There's a range of issues, from environment to health to education, where science is critical. I hope the public will become aware of the importance that the correct use of science in policy has on people's lives, that this will have an impact in the appropriate use of science by our government leadership.

Q: Last month, you joined the Project on Scientific Knowledge and Public Policy at George Washington University in Washington, D.C. Are you better able to initiate change being outside FDA?

Oh dear. [Laughing.] Well, I don't know that I can. Right now, many of the innovative ideas on how to address these issues at FDA [are] coming from outside the agency. We'll see what happens.

Q: Thinking about your activism makes us wonder: Do you feel like a superhero?

I don't. To my mind, my resignation was a small thing. But if it helped give energy to others, then that is well worth it.

Open to

Agilent's GeneSpring software puts your genomics data in biological context

Genomic data sets are often difficult to interpret. With GeneSpring—the industry standard in genomic data analysis—you can confidently uncover the biological processes that lie behind your experimental results. The GeneSpring software application suite enables you to quickly compare data from a variety of different applications based on your area of biological interest, opening the door to a systems-level approach. Whether analyzing large data sets, multiple data types, or data stored in different silos, GeneSpring helps you transform data into discoveries.

To hear from researchers who are charting their own course in Genomics **visit www.OpenGenomics.com**

Agilent Technologies

LETTERS

edited by Etta Kavanagh

Post-Fire Logging Debate Ignores Many Issues

RECENT CONTROVERSY CONCERNING POST-FIRE LOGGING IN OREGON is emblematic of the problems of "salvage logging" globally (*1*). Although tree regeneration after disturbances in forested areas is important (*2*–*4*), a narrow view of this issue ignores important ecological lessons, especially the role of disturbances in diversifying and rejuvenating landscapes. Scientific advances in recent decades demonstrate that disturbances are not catastrophes, trees in these landscapes are not wasted if they are not harvested, and post-fire logging is not forest restoration (*5*).

Fires (*6*), floods (*7*), volcanic eruptions (*8*), hurricanes (*9*), and insects (*9*) create and sustain the structure and composition of forests; disturbed areas also support species that are rare or absent from closed-canopy forests, including many that are restricted to recently burned areas (*6*). The extraordinary habitat mosaics of southwest Oregon's Biscuit fire area (*10*) and characteristic post-disturbance communities present in forests throughout the world (*11*) are in large part due to periodic "catastrophic" disturbances. Relative to naturally disturbed forests, intensively managed forests and plantations lack biological legacies, including intact understory vegetation, snags (standing dead trees) and logs, and patches of undisturbed or partially disturbed forest (*11*). Additionally, the heterogeneity associated with natural disturbances typically includes areas of low tree density and high shrub cover (*12*), which results in structural complexity required by many elements of the forest biota (*13*).

Two views of forests after the Biscuit fire of 2002 in the Siskiyou National Forest, southwest Oregon: (left) unlogged botanical reserve with legacy trees present and (right) adjacent logged area with legacy trees removed and soils damaged (blackened areas) by burning of logging slash. [Photos taken November 2005.]

CREDIT: R. SKAR

Ecological damage caused by post-disturbance logging may outweigh short-term economic benefits. If conducted improperly, timber harvest of any kind damages soils and below-ground processes, spreads invasive species, increases sediment delivery to streams, and destroys or degrades key environments for terrestrial and aquatic species. With post-disturbance logging, however, these impacts occur when forest recovery is most vulnerable to the effects of additional, especially anthropogenic, disturbances, creating cumulative effects not associated with logging in undisturbed forests (*14*, *15*). Such effects can extend for a century or more, because of the removal of long-persisting and functioning biological legacies (*11*). Moreover, a focus on post-disturbance logging will divert the attention of forest managers from conducting legitimate fuels reduction in fire-prone areas by, for example, thinning overly stocked trees and undergrowth, especially within at-risk rural communities, thereby exacerbating the already existing problem of declining local agency staffing and budgets.

The effects of post-disturbance logging require careful consideration of whether to log at all, and if so, how to conduct such logging to minimize negative consequences. If we must conduct post-disturbance logging for timber production, stringent ecological safeguards must be in place to minimize impacts to terrestrial (*14*) and aquatic (*15*) ecosystems. When viewed through an ecological lens, a recently disturbed landscape is not just a collection of dead trees, but a unique and biologically rich environment that also contains many of the building blocks for the rich forest that will follow the disturbance.

DOMINICK A. DELLASALA,[1*] JAMES R. KARR,[2] TANIA SCHOENNAGEL,[3] DAVE PERRY,[4] REED F. NOSS,[5] DAVID LINDENMAYER,[6] ROBERT BESCHTA,[7] RICHARD L. HUTTO,[8] MARK E. SWANSON,[9] JON EVANS[10]

[1]National Center for Conservation Science and Policy, Ashland, OR 97520, USA. [2]Professor Emeritus, University of Washington, Seattle, WA 98195, USA. [3]David H. Smith Research Fellow, Department of Geography, University of Colorado, Boulder, CO 80309, USA. [4]Professor Emeritus, Ecosystem Studies, Department of Forest Science, Oregon State University, Corvallis, OR 97331, USA. [5]Davis-Shine Professor of Conservation Biology, University of Central Florida, Orlando, FL 32816, USA. [6]Centre for Resource and Environmental Studies, The Australian National University, Canberra, ACT 0200, Australia. [7]Professor Emeritus, College of Forestry, Oregon State University, Corvallis, OR 97331, USA. [8]Avian Science Center, Division of Biological Sciences, University of Montana, Missoula, MT 59812, USA. [9]Forest Ecosystem Analysis, College of Forest Resources, University of Washington, Seattle, WA 98195, USA. [10]Landscape Analysis Laboratory, Department of Biology, University of the South, Sewanee, TN 37383, USA.

*To whom correspondence should be addressed. E-mail: Dominick@nccsp.org

References

1. D. B. Lindenmayer *et al.*, *Science* **303,** 1303 (2004).
2. M. Newton *et al.*, *Science* **313**, 615a (2006).

3. B. N. Baird, *Science* **313**, 615b (2006).
4. D. C. Donato *et al.*, *Science* **313**, 615c (2006).
5. R. F. Noss, D. B. Lindenmayer, *Conserv. Biol.* **20**, 946 (2006).
6. R. L. Hutto, *Conserv. Biol.* **9**, 1041 (1995).
7. M. Parsons, C. A. McLoughlin, K. A. Kotschy, K. H. Rogers, M. W. Rountree, *Front. Ecol. Environ.* **3**, 487 (2005).
8. C. M. Crisafulli, F. J. Swanson, V. H. Dale, in *Ecological Responses to the 1980 Eruption of Mount St. Helens*, V. H. Dale, F. J. Swanson, C. M. Crisafulli, Eds. (Springer Science, New York, 2005), pp. 287–299.
9. D. R. Foster, D. A. Orwig, *Conserv. Biol.* **20**, 959 (2006).
10. A. DellaSala, S. B. Reid, T. J. Frest, J. R. Strittholt, D. M. Olson, *Nat. Areas J.* **19**, 300 (1999).
11. D. B. Lindenmayer, J. F. Franklin, *Conserving Forest Biodiversity: A Comprehensive Multiscaled Approach* (Island Press, Washington, DC, 2002).
12. P. N. Neitlich, B. McCune, *Conserv. Biol.* **11**, 172 (1997).
13. D. B. Lindenmayer, R. F. Noss, *Conserv. Biol.* **20**, 949 (2006).
14. R. L. Beschta *et al.*, *Conserv. Biol.* **18**, 957 (2004).
15. J. R. Karr *et al.*, *BioScience* **54**, 1029 (2004).

Preventing HIV/AIDS in Adolescents

I WAS PLEASED TO SEE THAT THE UNITED Nations Population Fund (UNFPA) is cooperating with the Interreligious Committee in Honduras without compromising its own principles, particularly as regards the effectiveness of condoms in fighting HIV/AIDS ("Mission possible: integrating the Church with HIV/AIDS efforts," J. Cohen, Special Section on HIV/AIDS: Latin America & Caribbean, 28 July, p. 482). UNFPA has taken on a special mandate to work with the world's staggering numbers of adolescents who need scientifically based information and the wherewithal to make responsible decisions.

In 2002, Lois Abraham and I started 34 Million Friends, a grassroots organization that raises money and awareness of UNFPA (*1*). I have witnessed UNFPA youth centers in Mali and Senegal where the young are enticed by sports and perhaps a cyber cafe and then are deluged with information and peer counseling about sexual matters. Lois has witnessed the same dedication toward AIDS prevention in Nicaragua. The Bush Administration has withheld $34 million from UNFPA every year since 2002 and touts "abstinence only" policies abroad, which do not take into account forced early marriage of girls to older, more sexually experienced men and often their need to trade sex for food or school tuition. The United States should fully support the UNFPA in its human rights–based work for sexual health. UNFPA works in 140 countries at their invitation. Last year, 171 countries contributed to UNFPA, but not the United States. For shame!

JANE ROBERTS

Redlands, CA, USA.

Reference

1. See www.34millionfriends.org.

On Purpose in Conservation

THE EMPHASIS ON THE PRESERVATION OF biodiversity as the objective of conservation ("Global biodiversity conservation priorities," T. M. Brooks *et al.*, Review, 7 July, p. 58) has three distressing faults.

First, species contain ecotypes that are unique to their locales. As the range of the species is restricted, ecotypes are lost and the functional integrity of the natural communities in that region suffers. Although the ecotypes may be reproducible over many generations from a population residual in a protected "hot spot," the reproduction is not guaranteed and is certain to be slow.

Second, the very best efforts in preserving species in parks will be defeated if we allow the environment to erode out from

under them. The issues are not simply climatic disruption, but also include physical, chemical, and biotic disruption.

Finally, the focus on biodiversity by well-financed and obviously influential scientists appears to be an authoritative statement that the needs of conservation are finite and can be met adequately by establishing parks to preserve species in hot spots. The fact is that these objectives are appropriate but completely inadequate and, presented without elaborated conditions, become distracting to the point of being misleading.

The objective of conservation is the preservation of a fully functional biosphere as the only human habitat. That entails preservation of the full range of genetic potential in species, the species in all of its intrinsic diversity. This argument presents a far more aggressive mission for conservation, one much closer to the objective recognized, at least nominally, by Brazil in preserving by law a high fraction of each land holding in forested regions as intact forest and by New York State's Adirondack Park, which embraces villages, towns, and businesses operating under special rules governing forested land over 6 million acres. Success also entails immediate implementation of the Framework Convention on Climate Change to stabilize the heat-trapping gas content of the atmosphere at levels safe for nature and for people. Conservation as a whole demands a new design on how to manage the world, not one based on parks alone, which are bound to fail.

GEORGE M. WOODWELL

Woods Hole Research Center, Woods Hole, MA 02543, USA.
E-mail: gmwoodwell@whrc.org

Response

WOODWELL'S DISTRESS APPEARS TO STEM from confusion about the objective, strategy, and scale of conservation addressed by our Review. As suggested by our title, our aim was to review biodiversity conservation as an objective, and prioritization as a strategy, at the global scale. First, other conservation objectives beyond biodiversity are also valid, such as cultural diversity (*1*) and ecosystem services (*2*). Happily, there are many synergies between these objectives and that of biodiversity conservation, because they have similar distributions and threats and can therefore harness similar conservation responses.

Second, Woodwell's assertion that conservation should represent the "preservation of the full range of genetic potential in species, the species in all of its intrinsic diversity" is in no way antagonistic to the strategy of prioritization, as others have mistakenly claimed (*3*). Representation is about conserving everything; prioritization is about what to conserve first (*4*).

Third, the scale of coverage of our review was global: which regions should be the first targets for flexible resources worldwide? Woodwell concentrates his criticism on the scale of individual parks; we agree with him that this is not the only scale at which biodiversity conservation must be implemented. At the broadest, planetary scale, tackling the effects of climate change (*5*) will require intergovernmental policy instruments to reduce greenhouse gas emissions (*6*). At intermediate scales, management needs to maintain the landscape/seascape-level ecological processes on which biodiversity depends (*7*). However, at the finer, pragmatic level of much current conservation implementation, clear targets for safeguarding individual sites of global biodiversity significance are essential. This is the case whether the appropriate conservation tactic is the establishment or better management of protected areas, or the implementation of other site-scale efforts.

The "Key Biodiversity Areas" approach, for instance, is being used to identify sites through local and national processes and ownership, but following global standards and criteria (*8*). This work uses two decades of experience in 170 countries in identifying "Important Bird Areas" (*9*) as a foundation to incorporate newly available comprehensive data for mammals, amphibians, and other taxa (*10*). Major efforts are now under way through the Species Survival Commission of IUCN (the World Conservation Union) to compile equivalent data sets for reptile, plant, marine, and freshwater biodiversity [e.g., (*11*, *12*)]. A particularly urgent subset of Key Biodiversity Areas are the 595 sites identified by the "Alliance for Zero Extinction" and endorsed by more than 60 biodiversity conservation organizations (*13*, *14*).

We respectfully refer Woodwell to the last four paragraphs of our paper, and references therein, for further discussion of these points.

T. M. BROOKS,[1,2,3] R. A. MITTERMEIER,[1] G. A. B. DA FONSECA,[1,4] J. GERLACH,[5,6] M. HOFFMANN,[1]

Letters to the Editor

Letters (~300 words) discuss material published in *Science* in the previous 6 months or issues of general interest. They can be submitted through the Web (www.submit2science.org) or by regular mail (1200 New York Ave., NW, Washington, DC 20005, USA). Letters are not acknowledged upon receipt, nor are authors generally consulted before publication. Whether published in full or in part, letters are subject to editing for clarity and space.

J. F. LAMOREUX,[3] C. G. MITTERMEIER,[1] J. D. PILGRIM,[7] A. S. L. RODRIGUES[5]

[1]Conservation International, Washington, DC 20036, USA. [2]World Agroforestry Centre (ICRAF), University of the Philippines, Los Baños, Laguna 4031, Philippines. [3]Department of Environmental Sciences, University of Virginia, Charlottesville, VA 22904, USA. [4]Departamento de Zoologia, Universidade Federal de Minas Gerais, Belo Horizonte, MG 31270, Brazil. [5]Department of Zoology, University of Cambridge, Cambridge CB2 3EJ, UK. [6]Nature Protection Trust of Seychelles, Post Office Box 207, Victoria, Mahé, Seychelles. [7]BirdLife International in Indochina, 4/209 Doi Can Street, Ba Dinh, Hanoi, Vietnam.

References

1. W. J. Sutherland, *Nature* **423**, 276 (2003).
2. R. Costanza *et al.*, *Nature* **387**, 253 (1997).
3. K. Schmidt, *Science* **274**, 916 (1996).
4. R. A. Mittermeier *et al.*, *Am. Sci.* **91**, 384 (2003).
5. C. D. Thomas *et al.*, *Nature* **427**, 145 (2004).
6. T. Flannery, *The Weather Makers* (Text Publishing, Melbourne, Australia, 2006).
7. R. M. Cowling, R. L. Pressey, M. Rouget, A. T. Lombard, *Biol. Conserv.* **112**, 191 (2003).
8. G. Eken *et al.*, *Bioscience* **54**, 1110 (2004).
9. BirdLife International, *State of the World's Birds 2004* (BirdLife International, Cambridge, UK, 2004).
10. J. E. M. Baillie *et al.*, *Global Species Assessment* (IUCN, Gland, Switzerland, 2004).
11. W. Darwall, K. Smith, T. Lowe, J.-C. Vié, *The Status and Distribution of Freshwater Biodiversity in Eastern Africa* (IUCN, Gland, Switzerland, 2005).
12. A. S. L. Rodrigues, J. D. Pilgrim, J. F. Lamoreux, M. Hoffmann, T. M. Brooks, *Trends Ecol. Evol.* **21**, 71 (2006).
13. T. H. Ricketts *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **102**, 18497 (2005).
14. D. S. Wilcove, *Living Bird* **25** (no. 3), 8 (2006).

TECHNICAL COMMENT ABSTRACTS

COMMENT ON "Pairing and Phase Separation in a Polarized Fermi Gas"

Martin W. Zwierlein and Wolfgang Ketterle

Partridge *et al.* (Reports, 27 January 2006, p. 503) reported pairing and phase separation in a polarized Fermi gas. We argue that it is not possible to distinguish the superfluid from the normal regimes in the presented data, or to discern which clouds were phase-separated. Some of the reported conclusions are inconsistent with recent experiments.

Full text at www.sciencemag.org/cgi/content/full/314/5796/54a

RESPONSE TO COMMENT ON "Pairing and Phase Separation in a Polarized Fermi Gas"

Guthrie B. Partridge, Wenhui Li, Ramsey I. Kamar, Yean-an Liao, Randall G. Hulet

Zwierlein and Ketterle fail to establish that trap anharmonicities or other objective mechanisms affect the conclusions of our report. Instead, they make the subjective assertion that our claims are not supported by the data. In emphasizing discrepancies between our results and theirs, they ignore potentially important differences in physical parameters. We stand by the statements and claims made in our report.

Full text at www.sciencemag.org/cgi/content/full/314/5796/54b

Vinton G. Cerf
Vice President and Chief Internet Evangelist
Google

Amy Wu
Computer Science Student
Stanford University

Benjamin Mako Hill
Research Assistant
MIT Media Laboratory

Maria Klawe
President
Harvey Mudd College

NEUROSCIENCE

A Case for the Moral Organ?

Michael R. Waldmann

Imagine a trolley whose brakes have failed is about to run over five men who work on the tracks. However, the trolley could be redirected onto a side track where only one worker would be killed. Is it morally right to throw the switch? How about a similar situation in which one worker could be thrown off a bridge onto the tracks where he would be killed but would save the five by stopping the train with his weight? Most people have different intuitions about these cases, although both describe cases in which five people could be saved by harming one. Moral dilemmas can be challenging. Is it permissible to remove life support when somebody is in an irreversible coma? Is collateral damage in Iraq acceptable? Should we deny expensive medical care to the elderly and instead use the money for curing young children? May I lie to my spouse to avoid hurting her? We often have fast gut reactions to these situations without being able to give coherent justifications.

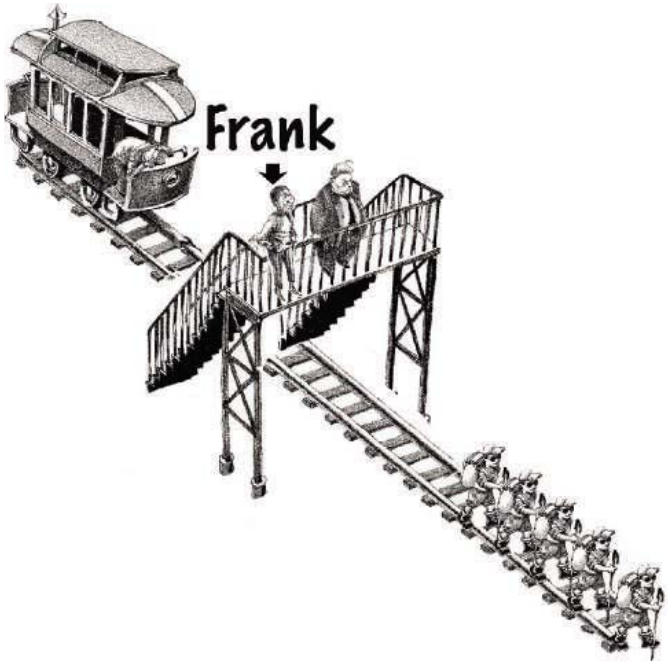
Moral Minds
How Nature Designed Our Universal Sense of Right and Wrong

by Marc D. Hauser

Ecco (HarperCollins), New York, 2006. 511 pp. $27.95, C$35.95. ISBN 0-06-078070-3.

If you are interested in where our intuitions in these and other moral situations come from, you must read Marc Hauser's *Moral Minds: How Nature Designed Our Universal Sense of Right and Wrong*. Written for a wide audience, the book provides a superb overview of one of the hottest topics in the life sciences. Hauser (a professor of psychology and director of the Cognitive Evolution Laboratory at Harvard University) presents a multitude of empirical studies on the cognitive foundations of moral intuitions and actions. He discusses moral cognition in adults, the development of moral competencies in infants and young children, and precursors of morality in animals.

Readers expecting only a discussion of studies narrowly focused on morality are in for a treat. Moral reasoning requires a large number of competencies, including the abilities to comprehend goal-directed actions, to distinguish between intended and merely foreseen side effects, to resist impulses and wait for gratifications, to understand what others are thinking or intending, to feel empathy, to cooperate, to deceive, and to feel emotions relevant for moral judgments (such as envy, pleasure, and pity). Hauser presents a balanced review of research on humans and nonhuman animals about all these topics in an eminently readable and scholarly way. He not only focuses on what is currently known but also points out limitations of the studies and sets goals for future research.



To save five, would you push one? Bystander Frank's dilemma.

CREDIT: TED DEWAN/FROM *MORAL MINDS*

The book's central aim is to provide answers to fundamental questions about the psychological and biological basis of morality: How does our brain generate moral intuitions? Do humans and other animals have an innate sense of morality? Or are moral behavior and moral reasoning based on learning?

Hauser begins the book with a bold theoretical claim: "we evolved a moral instinct, a capacity that naturally grows within each child, designed to generate rapid judgments about what is morally right or wrong based on an unconscious grammar of action." Of course, the author does not believe that we are born with specific moral rules (e.g., "do not cheat on your spouse"), because this would not explain why different cultures have created different moral systems. Rather, his theory draws from an analogy to linguistics. In the 1950s, the MIT linguist Noam Chomsky began developing the view that humans possess a "language organ" that contains a universal grammar. This grammar, in Chomsky's explanation, consists of universal syntactical rules and parameters that encode differences among languages. Learning the syntax of a specific language mainly involves setting the parameters of the universal grammar to the language-specific values. Using this theory as a blueprint for his own account, Hauser argues that we are endowed with an abstract universal moral grammar with parameters that encode cultural differences. [This argument has also been developed by John Mikhail in his doctoral dissertation (*1*) and a forthcoming book (*2*).] The moral grammar along with a variety of cognitive competencies underlies our morality.

Unfortunately, Hauser never explains what the rules and parameters of the moral grammar precisely look like. Findings that show that different cultures generate similar intuitions (as in the trolley problems above) are viewed as evidence for universal rules, whereas other studies showing huge cultural differences are interpreted as evidence for the role of parameters. This flexibility of the theory makes it hard to envision what could constitute a strict empirical test of the theory.

Indeed, many of the empirical studies that Hauser discusses could even be taken as evidence against the moral grammar view. The book is full of examples showing that different cultures have different notions of fairness. In most cultures harming other people is prohibited, but in some cultures female family members may be killed when they are suspected to be unchaste. Some societies allow their members to brutally kill the children of neighbor-

The reviewer is at the Department of Psychology, University of Göttingen, Gosslerstraße 14, 37073 Göttingen, Germany. E-mail: michael.waldmann@bio.uni-goettingen.de

ing tribes or to torture crime suspects. In the chapter on the trolley problem, Hauser argues that an abstract rule explaining our intuitions could be that harming people can be justified when the harmful act is not intended, but is rather a bad side effect of an act with good intentions. Again there are counterexamples: Some people feel that it is okay to hurt somebody's leg to save a life or that a physician should be held accountable for bad side effects that he did not intend. Hauser would probably argue that these are all parametric variations, but if so it is then hard to discern the underlying common abstract moral rule.

Although Hauser is not shy about his theoretical preferences, he presents alternative theories in a fair manner. One plausible alternative postulates cultural learning mechanisms (*3*) that draw on various general capacities, such as our abilities to understand other people as intentional agents and to imitate them—to mention just two of the capacities whose existence in children and some animals Hauser eloquently describes. Moral judgments need not be based entirely on reflective applications of explicit rules. They may draw on unconscious analogical reasoning, simple heuristics, and gut feelings. General cognitive mechanisms, such as attention, may also influence our judgments. For example, different causal representations of similar situations may highlight different aspects of the moral dilemmas (*4*).

Some of these components may be in part innate, although they need not necessarily be specific to the moral domain. Constraints on moral systems may also have historically developed as a result of cultural evolution. For example, it is hard to imagine a society that would survive if it created a moral system that punishes cooperation. Thus, there is certainly a place for both nature and nurture, and Hauser and his critics would agree that various competencies that are not specific to morality play an important role. Where they part company is on whether a dedicated cognitive system devoted to a moral grammar is also required (*5, 6*).

Regardless of how convincing Hauser's theory eventually proves, its boldness turns reading *Moral Minds* into a suspenseful experience. Near the end, Hauser reveals that he does not expect a definitive resolution soon and that he considers his theory a framework for future research rather than a summary of a finished project: "By leaning on the linguistic analogy, however, we open the door to these questions, and wait for the relevant theoretical insights and observations."

References

1. J. M. Mikhail, thesis, Cornell University, Ithaca, NY (2000).
2. J. M. Mikhail, *Rawls' Linguistic Analogy* (Cambridge Univ. Press, Cambridge, in press).
3. M. Tomasello, M. Carpenter, J. Call, T. Behne, H. Moll, *Behav. Brain Sci.* **28**, 675 (2005).
4. M. R. Waldmann, J. H. Dieterich, *Psychol. Sci.*, in press.
5. S. Nichols, in *The Innate Mind: Structure and Content*, P. Carruthers, S. Laurence, S. Stich, Eds. (Oxford Univ. Press, Oxford, 2005), pp. 353–369.
6. J. Prinz, in *Stich and His Critics*, D. Murphy, M. Bishop, Eds. (Blackwell, Malden, MA, in press).

10.1126/science.1132880

NEUROSCIENCE

The Powers of Rhythm

Pascal Fries

György Buzsáki's *Rhythms of the Brain* is an excellent compendium on the rapidly expanding research into the mechanisms and functions of neuronal synchronization. Buzsáki presents such synchronization as a binding glue that integrates many levels of neuroscientific investigation with one another and with neighboring disciplines. The text refers to more than a thousand articles and books. For many of these, the author provides a mini-review in a few sentences, and he summarizes selected references in informative figures. For this reason, the book might well have been subtitled "everything you ever wanted to know about how the brain works but never found the time to look up and read in the original literature."

Rhythms of the Brain
by György Buzsáki
Oxford University Press, Oxford, 2006. 464 pp. $69.50, £42. ISBN 0-19-530106-4.

All the same, the book is much more than a giant review. Buzsáki (a professor at Rutgers University's Center for Molecular and Behavioral Neuroscience) manages to elegantly integrate insights from physics, engineering, and cognitive psychology with contributions from cellular, systems, cognitive, and theoretical neuroscience. By connecting the pieces, he produces a whole that greatly exceeds the sum of its parts. His narrative begins and ends with neuronal synchronization, and he presents that as the key to understanding how everything comes together to make the brain work. The book suggests that Buzsáki has already arrived at integrative neuroscience, currently a much-sought target of funding and research organizations.

The book is organized into 13 chapters, which the author calls "cycles." The first introduces relevant general concepts such as periodicity and prediction. Here Buzsáki also presents his notion that most of the brain's activity is generated from within, whereas external inputs cause perturbations that are only minor albeit essential for rendering the brain's internal operations ecologically useful. He ends the chapter with an excellent summary of *Rhythms of the Brain*:

> The topics discussed in this book—emergence of spontaneous order, oscillations, synchrony, structure–function relationships, and representation and storage by cooperating cell assemblies—represent the middle grounds of brain activities between the microscopic mindless neurons and the wise, performing brain. My goal is to disclose how the brain gains its smartness from the organized complexity of its constituents. What follows is a progress report on the fascinating endeavors of neuroscience, a tour of fields that are usually not linked together in a single piece of scientific writing.

CREDIT: JOAN MARCUS

Behavior established through synchrony. "If you have seen Luis Bravo's Broadway extravaganza *Forever Tango*, you can picture the qualitative essence of neuronal synchrony: coupling through time by some invisible links."

The reviewer is at the F. C. Donders Centre for Cognitive Neuroimaging, Radboud University Nijmegen, Kapittelweg 29, Nijmegen 6525 EN, Netherlands. E-mail: pascal.fries@fcdonders.ru.nl

After exploring the principles that link structure to function, Buzsáki reviews the essentials of cortical microcircuitry, focusing on the important role that neuronal inhibition plays in functional diversity and the generation of rhythms. Introducing the methods currently used for measuring and analyzing rhythmic brain activity, the author discusses their relation to the underlying cellular and synaptic biophysics. He then presents the phenomenology of rhythms (from the circadian cycle to 600-Hz neuronal oscillations) and considers the fundamental scaling laws that connect them. The chapter "Synchronization by Oscillation" moves from the phenomenology to the underlying physiology and from oscillations to synchronization. Its revealing exploration of the mechanistic consequences of synchronization is one of the book's highlights.

Following discussions of sleep-related brain rhythms and the role of sleep rhythms for consolidating waking-time experiences, Buzsáki introduces gamma-band synchronization and the theories about its function. A chapter on perceptions and actions reviews the influence of brain states on brain rhythms and in turn on behavior. In "Oscillations in the 'Other Cortex,'" Buzsáki describes his "home turf": the hippocampus, its rhythms, and their putative functions for hippocampal coding and memory formation. He then turns to synchronization between hippocampus and neocortex and its putative function for coordination and for memory. Although many of the topics he addresses in these chapters have been reviewed extensively elsewhere, Buzsáki takes the discussions to a new level by assuming a bird's-eye perspective and integrating observations and concepts that have not previously been brought together. A final chapter offers some thoughts on a sample of remaining "tough problems," which range from the role of brain rhythms in the cerebellum and basal ganglia to their potential function in consciousness.

All of the chapters have a two-part structure: the main text and very extensive footnotes. Buzsáki reserves the former for an uninterrupted flow of thought. Although full of deep insights, this main text avoids technicalities and expert terminology; therefore, it should be understandable to any curious and open-minded reader. Through this approach, Buzsáki succeeds in following his credo that "discoveries and insights realize their power only when understood by others."

Even though the book is structured to reward nonspecialists, it is also a must-read for interested neuroscientists. My own research focuses on neuronal synchronization; nevertheless, I learned something from almost every paragraph. At some places, Buzsáki filled gaps in my knowledge; at others, he made me recognize overarching principles. For the expert neuroscientist, the footnotes add full scientific depth to *Rhythms of the Brain*. Branching from the main text, they provide extensive references, deepened discussions, and various historical anecdotes. There is bias toward Hungary and its scientists, but that becomes fully understandable reading this masterful book.

10.1126/science.1133986

NEUROSCIENCE

From the Dark Side to the Bright Side of Drug Addiction

Barry J. Everitt

Neurobiology of Addiction

by George F. Koob and Michel Le Moal

Academic Press (Elsevier), London, 2006. 502 pp. $129, £79.95. ISBN 0-12-419239-4.

The timely appearance of this ambitious book emphasizes how rapidly the neurobiology of addiction has emerged and grown as a research field. Some 30 years ago, we knew almost nothing about the neurobiological mechanisms of addiction. The rapid advances in our understanding owe much to the important interplay between animal experimentation and the scientific study of drug addiction in humans. Studies in humans, which allow measurements of subjective experience, have been greatly facilitated by the advent of functional imaging techniques. The text's authors, George Koob and Michel Le Moal, have made major contributions, both experimental and conceptual, to the field. In *Neurobiology of Addiction*, they review how we have come to understand the specific molecular mechanisms underlying the effects of the major classes of addictive drugs. They describe the cellular and molecular adaptations to chronic self-administration of drugs and discuss the neural systems that mediate the effects of addictive drugs as well as their withdrawal. The authors also outline the learning mechanisms engaged (perhaps aberrantly) by these drugs.

Koob and Le Moal first met in the late 1970s in Koob's lab at the Salk Institute in La Jolla, California; there soon followed a reciprocal visit by Koob to Le Moal in Bordeaux. *Neurobiology of Addiction* is a fitting culmination to the productive, long-term collaboration that grew out of these initial exchanges. This substantial volume succeeds both as a reference work—with chapters devoted to each of five major classes of drug (psychostimulants, opioids, alcohol, nicotine, cannabinoids)—and as a state-of-the art review of theories of drug addiction. The five chapters on drugs repeat a common structure, which makes them easy to follow. Once one gets used to it, the authors' approach provides a very helpful framework within which to consider the commonalities and differences among these classes of powerfully addictive drugs.

Koob made some of the fundamental observations showing that the mesolimbic dopamine neurons that innervate the nucleus acumbens mediate the acute reinforcing effects of addictive drugs. Collaborating with Le Moal, he subsequently focused on the "opponent motivational processes" through which addiction is hypothesized to originate (that is, through avoidance of the aversive effects of drug withdrawal). The authors developed their theory in the context of Richard Solomon's earlier ideas (*1*), which had suggested that drugs like heroin

CREDIT: IRA KORMAN/IMAGES.COM/CORBIS

The reviewer is in the Department of Experimental Psychology, University of Cambridge, Downing Street, Cambridge CB2 3EB, UK. E-mail: bje10@hermes.cam.ac.uk

caused a rapidly occurring, affectively hedonic ("pleasurable") "a" process. They argued that it is succeeded by a slower onset but longer-lasting "b" process (of opposite affective sign) that is a manifestation of withdrawal. In a key advance, Koob and Le Moal and their colleagues (including Athina Markou) defined the b process not in terms of the physical concomitants of withdrawal (which until then had been a key diagnostic feature of addiction) but as an aversive motivational state. This state is seen experimentally as raised reward thresholds [measured with intracranial self-stimulation (*2*)] and revealed behaviorally as conditioned aversive responses.

The authors' subsequent research has defined the neurochemical and molecular correlates of this opponent b process, which they have called "the dark side" of drug addiction. These include counteradaptations in the brain's reward system (characterized by decreased, rather than increased, dopamine and serotonin in the nucleus accumbens) as well as increased activity in the brain's stress and what they have termed its "anti-reward" systems. The latter is characterized by increased corticotropin-releasing factor and dynorphin within the extended amygdala, a neuroanatomical construct introduced by George Alheid and Lennart Heimer and embraced enthusiastically by Koob and Le Moal. More recently, these ideas have been augmented by applying to drug addiction the concept of "allostasis," a "state of chronic deviation of the regulatory system from its normal (homeostatic) operating level." In this view, repeated drug taking leads to (doomed) attempts by the brain to maintain stability through counteradaptations in its reward system and recruitment of antireward or stress systems. The "residual deviation from normal brain reward threshold regulation is termed an *allostatic state*"—an altered hedonic set point that cannot be normalized by taking more drug.

The authors have never been shy in vigorously promoting their allostatic theory of addiction, but then other theorists have often been equally robust in promoting theirs. Thus the balanced and appreciative way in which Koob and Le Moal consider the other contending theories is both refreshing and rewarding. In revealing the commonalities among many current theoretical approaches, they perhaps miss a trick in not fully considering them within the framework of the extended amygdala. And they cannot disguise their antipathy to the highly influential "incentive sensitization" theory of Terry Robinson and Kent Berridge, arguing that the unquestionable occurrence of behavioral sensitization not only is biased by the focus on stimulant drugs but also does not easily explain the escalation of drug intake that characterizes addiction. Robinson would no doubt strongly contest the assertion that rats that have escalated their drug intake are not sensitized—but the book may have gone to press before Robinson's recent paper showing sensitized motor stereotypy in escalated subjects (*3*) was published.

Readers will find Koob and Le Moal provide more than a reference source on neurobiological mechanisms and a review of current theories of addiction that champions their own. The first chapter offers a consideration of various definitions of addiction, which includes an attempt to explain the continuing and still puzzling use of "substance dependence" rather than "addiction" in the fourth edition of the *Diagnostic and Statistical Manual of Mental Disorders* (*DSM-IV*). The authors' discussion of different animal models of addiction is especially valuable because they address head on the definition of addiction when applied to animals and note that modeling this state requires chronic and probably escalating self-administration of the drug. That important aspect of drug addiction forms the central point of the final, engrossing chapter, which pits allostatic and incentive sensitization theories against one another. There the authors also consider the problem of vulnerability and the way that variation in the propensity to escalate drug intake might interact with the consequences of chronic drug taking (e.g., the impairment of frontal lobe executive functions) to result in compulsive drug seeking.

Here there arises a paradox that the authors do not address directly: Elegant work by Pier Vincenzo Piazza, Le Moal, and colleagues has shown that some animals within a population of laboratory rats have the propensity (presumably genetically determined) to self-administer very low doses of drugs whereas others do not, and it has been argued that this models the vulnerability to addiction. But subsequent research by Piazza and colleagues has shown that this behavioral trait does not predict the development of compulsive drug seeking after chronic drug self-administration, as measured by its persistence in the face of adverse circumstances such as punishment (*4*, *5*). (Such criteria are a diagnostic feature of substance dependence in *DSM-IV* and the *International Classification of Diseases, 10th edition*.) Clearly, much remains to be done to explore the validity of models of vulnerability to addiction.

The final chapter also presents Koob and Le Moal's "world view"of addiction, a step toward consensus theorizing that is to be applauded.

Neurobiology of Addiction is a major achievement and will rapidly become a must-have book on the shelves of addiction researchers. It is pleasingly free of errors (but it would be nice to know what Figure 3.13 should be). Although it includes much with which readers will disagree and argue, there is also much to relish in Koob and Le Moal's thought-provoking and scholarly text.

References

1. R. L. Solomon, *Am. Psychol.* **35**, 691 (1980).
2. A. Markou, G. F. Koob, *Neuropsychopharmacology* **4**, 17 (1991).
3. C. R. Ferrario *et al.*, *Biol. Psychiatry* **58**, 751 (2005).
4. V. Deroche-Gamonet, D. Belin, P. V. Piazza, *Science* **305**, 1014 (2005).
5. L. J. M. J. Vanderschuren, B. J. Everitt, *Science* **305**, 1017 (2005).

10.1126/science.1129565

NEUROSCIENCE

How Does the Brain Deal with the Social World?

Sarah Jayne Blakemore

In a very short time, social neuroscience has come from almost nothing to be one of the most flourishing research topics in neurobiology. Over the last five years, several neuroscience journals have devoted special issues to the biological underpinnings of social behavior. This past year has seen the launch of two specialist journals that cover the neural basis of social and emotional behaviors (*Social Neuroscience* and *Social Cognitive and Affective Neuroscience*). The increasing interest in the field is captured in MIT Press's Social Neuroscience series of edited volumes. For *Social Neuroscience: People Thinking About Thinking People*, the third title in the series, editors John Cacioppo, Penny Visser, and Cynthia Pickett have recruited contributors from a range of disciplines (including psychology, psychiatry, neurology, radiology, and neuroscience) to focus on social behavior and cognition in humans.

One of the volume's central questions is whether dedicated cognitive and neural

The reviewer is at the Institute of Cognitive Neuroscience, University College London, 17 Quenn Square, London WC1N 3AR, UK. E-mail: s.blakemore@ucl.ac.uk

mechanisms exist for understanding and interacting with other people. As Jason Mitchell and his colleagues remark in their chapter, "In being governed by complex mental states, other people are a unique kind of stimulus." The statement is supported by neuroimaging studies that show the brain is activated differently when subjects make judgments about a human than when they make the same judgments about inanimate objects. The brain areas involved in making judgments about a human overlap with those involved in thinking about other people's mental states (i.e., involved in what is known as "theory of mind") (*1*). One of these brain regions is the medial prefrontal cortex, a large area whose exact role in social cognition is elusive (*2*). Further research by the Mitchell group cast doubt on the idea that the role of the medial prefrontal cortex is simply to think about people. In a second study, the region was activated whenever subjects made mental state judgments, whether about a human or an animal.

Guys in a group.

Social Neuroscience
People Thinking About Thinking People

John T. Cacioppo, Penny S. Visser, and Cynthia L. Pickett, Eds.

MIT Press, Cambridge, MA, 2006. 320 pp. $45, £29.95. ISBN 0-262-03335-6. Social Neuroscience.

In a chapter on the differential roles of brain regions (including the medial prefrontal cortex) that are activated in theory of mind tasks, Rebecca Saxe reevaluates existing research on theory of mind. She suggests that a different part of the network, the temporo-parietal junction, is the more critical region implicated in reasoning about mental states. Another hypothesis on the medial prefrontal cortex's function is put forward by Debra Gusnard, who discusses activation of the region in "rest" conditions and in tasks in which subjects think about the self. Disentangling methodological problems of theory of mind research, Valerie Stone concludes that the executive function demands of theory of mind tasks might induce activity in the medial prefrontal cortex. Although the reader is not left with a single compelling and indisputable theory, the divergence among the explanations of medial prefrontal cortex function illustrates that the contributions to the book reflect the cutting edge of social neuroscience research.

Another question that the contributors return to throughout the volume is whether social cognitive processes are fundamentally different from general cognitive processes such as attention, perception, and memory. Ralph Adolphs asks, "Is social cognition a particular type of cognition?" Again, the answer is not clear cut and seems to depend on what aspect of social cognition we are talking about. Thinking about other people's mental states might be one area of social cognition that involves special mechanisms and cannot be explained by component cognitive processes. Whereas other aspects of thinking about people (for example, social exclusion and racial bias) may rely on mechanisms that are not unique to social cognition. Matthew Lieberman and Naomi Eisenberger describe their clever neuroimaging experiment in which subjects played a computerized ball game with two other "virtual" people. Under one condition, the two other players stopped throwing the ball to the subject. This social exclusion condition was associated with activity in the brain's pain network. Lieberman and Eisenberger argue that feeling the pain of being excluded by others involves the same mechanisms as those underlying physical pain. Three chapters focus on stereotype biases, including gender and racial prejudices. In one, Elizabeth Phelps and Mahzarin Banaji suggest that stereotype bias is a form of classical conditioning.

Investigating the neural mechanisms that underly complex social capacities is an ambitious remit and one that would not be possible without the ability to scan people's brains. Marcus Raichle quotes Ivan Pavlov: "If we could look through the skull into the brain of a consciously thinking person, and if the place of optimal excitability were luminous, then we should see playing over the cerebral surface, a bright spot with fantastic, waving borders constantly fluctuating in size and form." Neuroimaging has undoubtedly revolutionized cognitive neuroscience. Nonetheless, a recent paper by Cacioppo and his colleagues warns against using neuroimaging simply to show that there are neural correlates of social perception and behavior (*3*). It is an ongoing challenge to ensure that tasks and stimuli are both highly controlled and at the same time have some ecological validity. Even when these challenges are met, it is important that neuroimaging analyses do not stand alone. The advantages of taking multiple approaches are demonstrated by the study of emotion, which is briefly described in Ralph Adolphs's elegant chapter. In this field, our understanding has benefited from the application of many different methodologies.

Clearly, a relatively brief volume such as this cannot be exhaustive. One topic of social neuroscience research noticeably absent from the book is the mirror neuron system (*4*). The system, which includes premotor cortex and inferior parietal cortex in monkeys and humans, is activated by execution of action and by the mere observation of action. Recently, it has been proposed that the mirror neuron system plays a role in social cognition: in the decoding of others' actions and the understanding of the intentions, goals, and desires that lie behind the actions (*5*). Two other broad topics not discussed to any substantial extent in the book are disorders characterized by social impairment (such as autism and psychopathy) and the development of the social brain. I look forward to finding coverage of these topics in future volumes of the series. In the meantime, researchers and students will find *Social Neuroscience* a valuable introduction to the neurobiological foundations of interactions among humans.

References

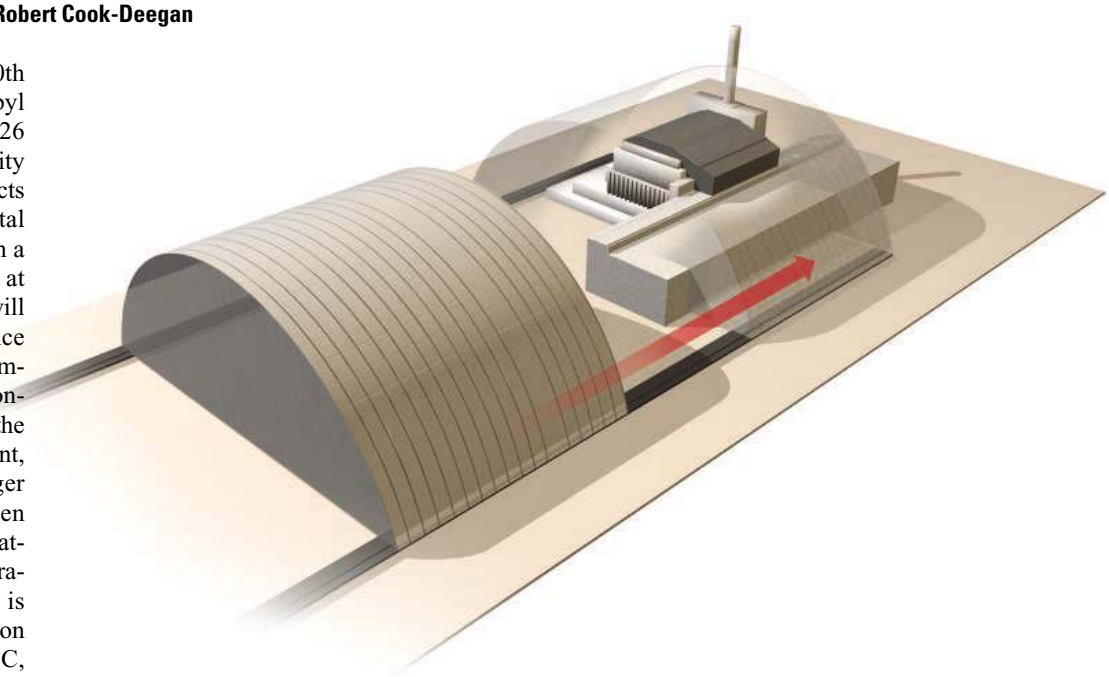
1. U. Frith, C. D. Frith, *Philos. Trans. R. Soc. London Ser. B* **358**, 459 (2003).
2. D. M. Amodio, C. D. Frith, *Nature Rev. Neurosci.* **7**, 268 (2006).
3. J. T. Cacioppo *et al.*, *J. Pers. Soc. Psychol.* **85**, 650 (2003).
4. G. Rizzolatti, L. Fogassi, V. Gallese, *Brain Res.* **1079**, 661 (2006).
5. V. Gallese, *Brain Res.* **1079**, 15 (2006).

10.1126/science.1133693

PUBLIC HEALTH

Genomics and Medicine at a Crossroads in Chernobyl

Geoffrey S. Ginsburg,* Misha Angrist, Robert Cook-Deegan



As workers begin construction of a new containment structure at the Chernobyl nuclear energy plant, research to study the effects of radiation exposure will benefit the Chernobyl community and the general public.

CREDIT: C. BICKEL/*SCIENCE*

Having recently observed the 20th anniversary of the Chernobyl nuclear reactor disaster of 26 April 1986, we now have an opportunity to mitigate and assess the health effects of radiation and other environmental exposures objectively and carefully in a controlled and longitudinal fashion at the same site. Thousands of workers will soon descend upon Chernobyl once again. The original 1.1-billion-ton temporary "sarcophagus" designed to contain the reactor site, as well as to limit the long-term consequences of the accident, is on its last legs: Its roof is no longer sealed, its structural integrity has been compromised, and the water accumulating under the reactor may be highly irradiated (*1*). The original structure is slated to be replaced by an $800 million New Safe Confinement shelter (NSC, also often called "the Ark") measuring 885 feet wide and 360 feet high. Construction is expected to commence soon and to be completed by 2010 (*2*).

Workers constructing the NSC and demolishing the residual nuclear power plant (under the auspices of the Shelter Implementation Plan or SIP) will be exposed to a number of risks, primarily ionizing radiation. These workers present a unique opportunity to examine the genome, environmental exposures, and their interaction and impact on human health. We believe it is imperative to establish a research and clinical framework to learn about human health effects of radiation during the construction of the Ark—first and foremost to protect Ukrainian workers, but also to manage future nuclear disasters.

What Do We Know Now About the Effects of Exposures at Chernobyl?

Last fall, the Chernobyl Forum, a consortium of eight United Nations agencies and the governments of Ukraine, Belarus, and Russia, released the most comprehensive report to date on the long-term health, environmental, and socioeconomic impacts of the Chernobyl disaster (*3*). Following explosion of the unit 4 reactor, Soviet military officials dispatched more than 600,000 rescue personnel, or "liquidators," to extinguish the fire in the reactor core, to consolidate radioactive waste, and to begin construction of the concrete and steel sarcophagus (*4*, *5*). The full health effects of the liquidators' exposure remain the subject of much debate. It is not clear, for example, whether Chernobyl cleanup workers have experienced an increase in hematological malignancies (*6*) or cancers of the thyroid, brain, and other solid tumors (*7*). Indeed, there has been no systematic, comprehensive evaluation—nor means to carry out such an evaluation—for those working and living at or near the site.

As summarized in the Chernobyl Forum report and elsewhere (*3*, *8*), epidemiological investigations of the consequences of Chernobyl radiation exposure, by definition, could not be carried out in a controlled fashion. Dosage, duration, and proximity were often crudely estimated. In addition, widespread ignorance of the effects of radiation and the imposition of a 2-year information blackout by the Soviet government hampered attempts to carry out an honest and open inquiry into radiation-related health outcomes (*5*, *8*).

Worker Safety and Other Ethical Issues

It is entirely possible, if not probable, that workers at the NSC site will be in close proximity to a number of sources of radioactivity, including (i) contaminated groundwater; (ii) radioactive waste that has been sitting in trench and landfill facilities; and (iii) substantial quantities of cesium-137, which has a half-life of 30 years (*3*, *9*). During NSC construction, remotely operated equipment will be deployed to minimize worker exposures. To its credit, the SIP provides for equipment designed to enhance worker safety (*9*). Because radiation exposure can be monitored closely with conventional dosimetry, it should be possible to minimize health risks. However, novel molecular technologies will be needed to provide a precise biologic measure of radiation dose and host reaction (*10*, *11*).

Any long-term initiative centered on Ark workers should have as its foremost goal the monitoring, maintenance, and improvement of worker health. Aggressive surveillance of premalignancies, malignancies, cataracts, cerebrovascular and cardiovascular diseases, immune dysfunction, and other known indicators of radiation exposure must be paramount. Ideally, workers at risk for higher lev-

The authors are with the Institute for Genome Sciences & Policy, Duke University, Durham, NC 27708, USA.

*Author for correspondence. E-mail: geoffrey.ginsburg@duke.edu

els of radiation exposure will have samples of their stem cells collected. In case those workers are exposed to myeloablative radiation doses [>400 cGy (centigray, absorbed dose of ionizing radiation) total], they could then be reinfused with their own peripheral blood stem cells (*12*).

Opportunities for Information Gathering and Scientific Inquiry

Assessing individual radiation responses among the 1400 workers per shift in the construction area (*9*) should be tremendously valuable for understanding the biology of low-level radiation exposure, as well as for developing novel biomarkers to monitor future radiation-intensive events, be they accidental, a consequence of war, or the result of terrorist activities. We envision measuring a broad range of biological markers of exposure before, during, and after the project, as well as health outcomes with which those markers can be correlated. Preliminary retrospective biomarker studies of Chernobyl cleanup workers have already correlated Chernobyl exposure with cytogenetic abnormalities (*13*).

To conduct a meaningful scientific inquiry, a research study and health care–delivery infrastructure must be created. A hospital in nearby Slavutich is set to provide basic safety training, medical screening, and radiation surveillance and monitoring (*9*), but a robust system needs to be established for collecting and storing reliable clinical data and for preserving biological samples. Data collection and tracking systems will be essential to handle hundreds of thousands of data points.

To establish baseline measures among the several thousand workers rotating through the site, essential clinical and demographic information should be collected at time zero and thereafter at regular intervals. A key component would be a guideline-compliant, privacy-protected biospecimen resource to facilitate the study of gene-environment interactions. Genomewide assessments have already provided detailed molecular phenotypes for ionizing radiation dosage (*14*) and other occupational exposures (*15*). Data from blood cells should offer critical insights into the hematopoietic and immune-system health of Ark workers. In particular, serial cytogenetic and gene-expression profiling of peripheral blood mononuclear cells will help define physiological and cellular changes resulting from radiation exposure (*16*). An important goal at Chernobyl will be to closely monitor and systematically correlate health status with promising biomarkers and to translate these findings into protective practices such that worker safety can be assured.

Exposure to ionizing radiation is well known to induce DNA damage in mammalian germ cells and can adversely affect those exposed and their offspring (*17*). Fertility and reproductive health monitoring are therefore likely to be crucial components of any large-scale study of Ark workers.

Cognition and mental health may be more important still (*3*, *18*). The relation between cognitive abilities and low-dose ionizing radiation is unclear; however, radiation is known to exert profound effects on the brain (*19*). Cognitive function should be measured in workers before exposure and regularly thereafter. Mental health and social dynamics can also be assessed to understand the factors that might contribute to fear, anxiety, depression, and other affective symptoms.

Considerations for the Future

This will be more than just a technical challenge, but a social, political, and ethical one as well. Jobs are scarce in the Chernobyl area, and workers will have incentives to take risks with their own health. Effective interventions to prevent radiation-related illnesses will need to link indicators of exposure to measures that reduce exposure, such as change of job or location, in a way that workers support and that is mindful of their best interests. Among the challenges of such a public health study will be reconciling individual workers' roles as employees with their status as research participants. At every stage, we would expect the guiding principle to be that a worker will be no worse off for having participated in the study.

In our view, the science needs to be tightly linked to health services, which is no mean task even in the best of circumstances in advanced economies. But most of all, the system should be constructed in a way that warrants workers' trust.

The issue of cost cannot be ignored. In light of the uncertainties surrounding radiation dosimetry, as well as the timeline and scope of the NSC construction job itself, it is difficult to pinpoint a bottom-line figure for an undertaking like the one we propose. We can imagine the sponsors of such a project working with Ukrainian public health officials and other regional institutions to develop a framework that includes a consensus limit on acceptable exposures such as the one advocated by the International Commission on Radiological Protection (50 millisieverts per year, which measures the biological effects of radiation) (*20*). It should then be possible to estimate the per-worker cost of averting higher cumulative doses a priori (*21*), while also providing for the construction of a lasting scientific infrastructure at the reactor site. If multiple nations are willing to collectively provide as much as $1 billion to secure Chernobyl for the next 100 years by building the Ark, surely they can also see the logic of supporting a once-in-a-lifetime scientific effort to produce an unprecedented knowledge base for a small fraction of the total construction costs. Moreover, even without the research benefits, worker health must be protected, and that will require new technologies.

Facilitating cutting-edge genomic research while deploying state-of-the-art public health measures in Chernobyl presents an extraordinary opportunity for science and medicine to clarify the impact of measured exposures on human health. It might further help quell anxiety in the event of future large-scale exposures to radiation both in the Ukraine and around the world, and without a 20-year learning curve.

References and Notes

1. M. D. Bellaby, "Crumbling Chernobyl shelter poses danger," *Associated Press,* 22 April 2006.
2. A. Krushelnycky, "Stalled: the Chernobyl rescue ark," *Sunday Times*, 23 April 2006.
3. The Chernobyl Forum, *Chernobyl's Legacy: Health, Environmental and Socio-economic Impacts* (International Atomic Energy Agency, Austria, 2005) (www.iaea.org/Publications/Booklets/Chernobyl/chernobyl.pdf), accessed 1 May 2006.
4. R. Stone, *Science* **312**, 180 (2006).
5. A. M. Kellerer, *Radiat. Environ. Biophys.* **41**, 1 (2002).
6. D. Gluzman *et al.*, *Hematol. J.* **5**, 565 (2005).
7. M. Rahu *et al.*, *Int. J. Cancer* **119**, 162 (2006).
8. M. Rahu, *Eur. J. Cancer* **39**, 295 (2003).
9. E. A. Schmieman, M. Wrona, P. Convert, D. Couch, *Nucl. Eng. Int.*, 6 April 2006 (www.neimagazine.com/story.asp?storyCode=2035380), accessed 17 May 2006.
10. V. K. Mazurik, V. F. Mikhailov [in Russian], *Radiats. Biol. Radioecol.* **44**, 296 (2004).
11. H. Braselmann *et al.*, *Mutat. Res.* **578**, 124 (2005).
12. F. Herodin, J. F. Mayol, F. Mourcin, M. Drouet, *Folia Histochem. Cytobiol.* **43**, 223 (2005).
13. E. Neronova, N. Slozina, A. Nikiforov, *Radiat. Res.* **160**, 46 (2003).
14. N. Touil, P. V. Aka, J. P. Buchet, H. Thierens, M. Kirsch-Volders, *Mutagenesis* **17**, 223 (2002).
15. M. T. Smith *et al.*, *Chem. Biol. Interact.* **153-154**, 123 (2005).
16. M. A. Coleman *et al.*, *Radiat. Res.* **164**, 369 (2005).
17. P. Jacquet, *J. Biol. Regul. Homeost. Agents* **18**, 106 (2004).
18. J. M. Havenaar *et al.*, *Am. J. Psychiatry* **154**, 1605 (1997).
19. G. Silasi *et al.*, *Biochem. Biophys. Res. Commun.* **325**, 1223 (2004).
20. International Commission on Radiological Protection (ICRP), "The optimisation of radiological protection" (Revision 25, ICRP, Stockholm, Sweden, 11 April 2005) (www.icrp.org/docs/Optimisation_web_cons_draft_42_105_05.pdf), accessed 16 May 2006.
21. K. Eged *et al.*, *Health Phys.* **80**, 137 (2001).

22. We thank H. F. Willard for helpful comments and suggestions.

10.1126/science.1130274

CELL SIGNALING

The Double Life of a Transcription Factor Takes It Outside the Nucleus

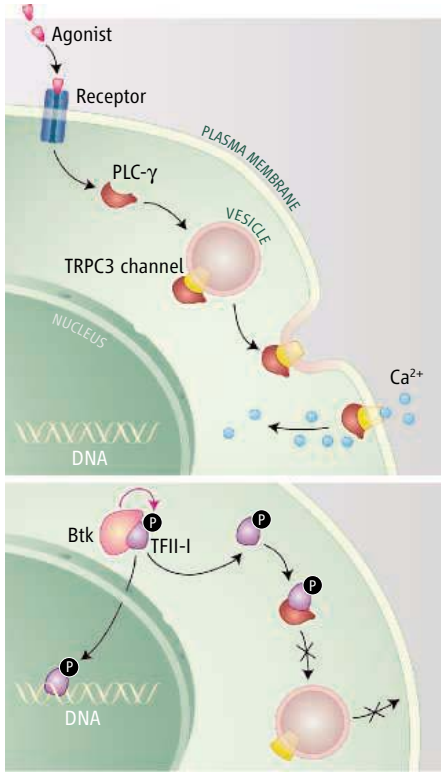
Although transcription factors had been thought to act only in the nucleus, new evidence that they can regulate calcium channels in the cytoplasm represents a mechanism for two-way communication within cells.

Chan Young Park and Richard Dolmetsch

It has long been known that calcium channels activate cellular signaling pathways that regulate transcription factors, but on page 122 of this issue, Caraveo *et al.* (*1*) turn this signaling paradigm on its head. They report that TFII-I, a ubiquitously expressed transcription factor, regulates the activity of TRPC3, a cell surface Ca^{2+} channel. Remarkably, the transcription factor affects the Ca^{2+} channel not through its effects on gene expression, but by competing with the channel for binding the enzyme phospholipase C (PLC). This mechanism of channel regulation reveals an entirely new way by which the TFII family of transcription factors can control cellular physiology and development. TFII-I thus joins a small group of transcription factors that function in both the nucleus and the cytoplasm.

TFII-I belongs to a family of general transcription factors, three of which are found in the 7q11.23 chromosomal region that is deleted in the congenital developmental disorder Williams-Beuren syndrome (*2*). Symptoms of this disease include hypersociability, mental retardation, and cardiac anomalies. The cardiac deficiencies are probably caused by deletion of the *elastin* gene (*3*), but the neurological and psychiatric symptoms might be due to deletion of neighboring genes such as *TFII-I* (*4*). TFII-I regulates transcription both by constitutively binding to the core sequence (Inr) found in the minimal promoter of most genes and by binding to other regulatory sites that lie in gene enhancers (*5*). Intriguingly, TFII-I is phosphorylated by Bruton's tyrosine kinase (Btk) (*6*), a protein that is important for B lymphocyte activation and which is mutated in X-linked agammaglobulinemia.

Agonist-induced activation of certain cell surface receptors is coupled to the activation of PLC, which leads to the release of Ca^{2+} from intracellular stores and Ca^{2+} influx across the plasma membrane (*7*). The resulting rise in intracellular Ca^{2+} is essential for many cell functions. Agonist-controlled Ca^{2+} entry depends on several types of ion channels including members of the transient receptor potential (TRP) family (*8*). TRPC3, the channel studied by Caraveo *et al.*, has been implicated in axon guidance (*9*) and in the development of cardiac hypertrophy (*10*).



Two jobs. (**Top**) Upon activation of specific receptors at the cell surface, PLC-γ binds to TRPC3 channels, causing insertion of the channels into the plasma membrane, where they mediate Ca^{2+} entry. (**Bottom**) The kinase Btk phosphorylates the transcription factor TFII-I, causing it to dissociate from Btk and bind to PLC-γ as well as enter the nucleus. By preventing PLC-γ from binding to TRPC3, TFII-I reduces the number of TRPC3 channels at the cell surface and inhibits agonist-controlled Ca^{2+} entry.

How PLC activates TRPC3 is somewhat controversial. PLC normally acts by cleaving phosphatidylinositol 4,5-bisphosphate to generate inositol trisphosphate and diacylglycerol. However, the authors previously reported that PLC-γ activates TRPC3 independently of its enzymatic activity (*11*) by binding to TRPC3 and increasing channel insertion into the plasma membrane. Among the five PLC subfamilies, PLC-γ members have a unique structure consisting of two separated halves of a pleckstrin homology (PH) domain, a conserved lipid-binding motif. Receptor activation causes the C-terminal half of the PH domain in PLC-γ to bind a PH-like "half domain" in TRPC3, thus increasing the plasma membrane insertion of TRPC3 and increasing Ca^{2+} influx (*12*).

Hints of a functional link between TFII-I and the TRPC3 channel came from the discovery that TFII-I binds the TRPC3 regulator, PLC-γ. When phosphorylated by Btk, TFII-I binds to a Src homology 2 (SH2) domain on PLC-γ. In addition, TFII-I binds to PLC-γ through an interaction between the partial PH domains in TFII-I and PLC-γ. Because the PH domain of PLC-γ is important for regulating TRPC3 at the cell surface, the authors investigated whether TFII-I expression regulates TRPC3 function. They found that reducing TFII-I protein levels increases Ca^{2+} influx whereas TFII-I overexpression reduces it, suggesting that TFII-I is a negative regulator of agonist-controlled Ca^{2+} entry. Deletion of a nuclear localization sequence did not affect the ability of TFII-I to reduce agonist-controlled Ca^{2+} entry, indicating that the transcription factor's function in the cytoplasm and nucleus are independent of each other.

Because PLC-γ regulates agonist-controlled Ca^{2+} entry by controlling the amount of TRPC3 at the cell surface, Caraveo *et al.* determined whether TFII-I regulates TRPC3 insertion into the plasma membrane by binding to PLC-γ. Reduced expression of TFII-I increased TRPC3 at the cell surface whereas overexpression of TRPC3 had the opposite effect. This depended on the partial PH domains in TFII-I and PLC-γ. Thus, cytoplasmic TFII-I can bind to the PH domain of PLC-γ and prevent PLC-γ from causing TRPC3 insertion in the plasma membrane.

So how does the interaction of TFII-I and PLC-γ regulate the physiology of cells? In Williams-Beuren syndrome, reducing the amount of TFII-I and its closely related family members might lead to overactivity of TRPC3, to errors in axon pathfinding, and to other developmental defects that depend on receptor-controlled calcium entry. Mutations of the voltage-activated L-type Ca^{2+} channel are associated with autism (*13*), suggesting that Ca^{2+} signaling can cause subtle effects on nervous system development that can result in cognitive disorders.

The study by Caraveo *et al.* raises several

The authors are in the Department of Neurobiology, Stanford University School of Medicine, Stanford, CA 94305, USA. E-mail: ricardo.dolmetsch@stanford.edu

CREDIT: P. HUEY/SCIENCE

interesting questions. How is the relative abundance of TFII-I in the cytoplasm and nucleus determined? TFII-I phosphorylation causes TFII-I translocation into the nucleus (*14*), and yet this phosphorylated form of the protein also binds to PLC-γ in the cytoplasm. Understanding precisely how these two pools of TFII-I are regulated will reveal how the two functions of the molecule are controlled. PLC-γ also plays a key role in activating many signaling enzymes, including protein kinase C, and TFII-I may regulate many of these signaling events at the plasma membrane. The existence of proteins such as TFII-I and DREAM/KChIP (*15*, *16*) that regulate both transcription and ion-channel function support an emerging paradigm whereby proteins that function both in the nucleus and in the cytoplasm of cells coordinate the overall ability of a cell to respond to membrane stimuli and to activate gene expression.

References

1. G. Caraveo, D. B. van Rossum, R. L. Patterson, S. H. Snyder, S. Desiderio, *Science* **314**, 122 (2006).
2. Y. Franke, R. J. Peoples, U. Francke, *Cytogenet. Cell Genet.* **86**, 296 (1999).
3. A. K. Ewart *et al.*, *Nat. Genet.* **5**, 11 (1993).
4. S. K. Danoff, H. E. Taylor, S. Blackshaw, S. Desiderio, *Neuroscience* **123**, 931 (2004).
5. A. L. Roy *et al.*, *EMBO J.* **16**, 7091 (1997).
6. W. Yang, S. Desiderio, *Proc. Natl. Acad. Sci. U.S.A.* **94**, 604 (1997).
7. G. S. Bird *et al.*, *Curr. Mol. Med.* **4**, 291 (2004).
8. P. D. Wes *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **92**, 9652 (1995).
9. Y. Li *et al.*, *Nature* **434**, 894 (2005).
10. H. Nakayama, B. J. Wilkin, I. Bodi, J. D. Molkentin, *Faseb J.* **20**, 1660 (2006).
11. R. L. Patterson *et al.*, *Cell* **111,** 529 (2002).
12. D. B. Van Rossum *et al.*, *Nature* **434**, 99 (2005).
13. I. Splawski *et al.*, *Cell* **119**, 19 (2004).
14. C. D. Novina *et al.*, *Mol. Cell. Biol.* **19**, 5014 (1999).
15. W. F. An *et al.*, *Nature* **403**, 553 (2000).
16. A. M. Carrion, W. A. Link, F. Ledo, B. Mellstrom, J. R. Naranjo, *Nature* **398**, 80 (1999).

10.1126/science.1133757

CHEMISTRY

Mass Spectrometry: Bottom-Up or Top-Down?

A novel approach to mass spectrometry involving fragmentation of intact proteins in the gas phase promises to greatly improve our ability to determine protein modifications.

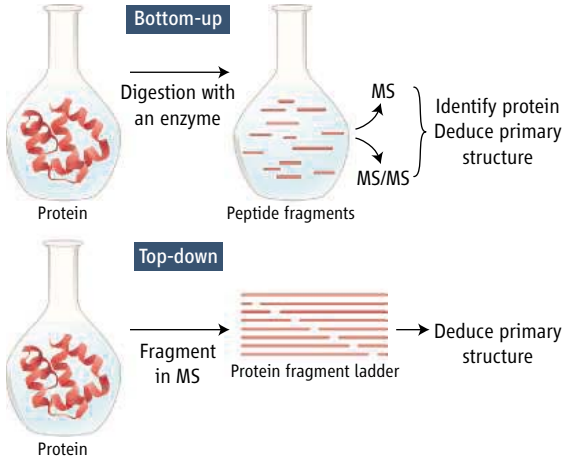
Brian T. Chait

The current revolution in proteomics and systems biology is driven by new analytical tools that are both fast and sensitive. Among these tools, mass spectrometry has become the method of choice for rapidly identifying proteins and determining details of their primary structures (*1*). Currently, there are two complementary lines of attack for the mass spectrometry analysis of proteins: the bottom-up and top-down approaches. On page 109 of this issue, Han *et al.* (*2*) extend the range of the top-down approach to proteins with molecular masses as high as 229 kD.

The bottom-up approach (see the figure, top panel) is widely used for identifying proteins and determining details of their sequence and posttranslational modifications (*1*). In this approach, proteins of interest are digested with an enzyme such as trypsin, and the resulting "tryptic peptides" are analyzed by electrospray ionization (ESI) or matrix-assisted laser desorption/ionization (MALDI). These mass spectrometry techniques allow peptide and protein molecular ions to be put into the gas phase without fragmentation (*3*). The ESI– or MALDI–mass spectrometry analyses take place in two stages. First, the masses of the intact tryptic peptides are determined; next, these peptide ions are fragmented in the gas phase to produce information on their sequence and modifications.

The bottom-up approach is especially useful for identifying proteins, because tryptic peptides are readily solubilized and separated, tasks that are considerably more difficult for the parent proteins. In addition, many tryptic peptides can be readily analyzed by mass spectrometry analysis, providing useful fragmentation ladders (*4*) that often yield sufficient information to identify the parent protein. Unfortunately, only a small fraction of the tryptic peptides are normally detected, and only a fraction of these yield useful fragmentation ladders. The bottom-up approach is therefore suboptimal for determining modifications and alternative splice variants (*5*). It is a little like having a jigsaw puzzle, where many of the pieces are missing.

But even if we had all the pieces, the picture would still be incomplete, because—to produce a sufficient number of tryptic peptide ions to allow for their detection by mass spectrometry—it is currently necessary to examine the pieces of a billion or more copies of the protein of interest. So really we have a billion jigsaw puzzles, some of which are the same, but many of which are slightly different, because they correspond to copies of the protein containing different modifications. Thus, if the pieces are relatively small (as they usually are for tryptic peptides), we will lose



Dissecting the primary structures of proteins by mass spectrometry. In the widely used bottom-up approach (**top**), proteins of interest are digested in solution with an enzyme such as trypsin, and the resulting peptides are analyzed in the gas phase by mass spectrometry in two stages. In the first (labeled "MS"), the masses of the intact tryptic peptides are determined; in the second (labeled "MS/MS"), these peptide ions are fragmented to produce information on the identity and sequence of the protein as well as its modifications. In the top-down approach (**bottom**), intact protein ions are introduced into the gas phase and are fragmented and analyzed in the mass spectrometer, yielding the molecular mass of the protein as well as protein ion fragment ladders; this information can be used to deduce the complete primary structure of the protein. Both methods make extensive use of correlations of the mass spectrometric data with protein and whole-genome sequence databases.

CREDIT: P. HUEY/*SCIENCE*

The author is in the Laboratory for Mass Spectrometry and Gaseous Ion Chemistry, Rockefeller University, New York, NY 10021, USA. E-mail: chait@rockefeller.edu

correlations that may exist between these modifications on disparate portions of the protein. Such correlations may exist, for example, on a subpopulation of the protein that carries a phosphate moiety at two or more sites simultaneously.

In the top-down approach (see the figure, bottom panel), intact protein ions are introduced into the gas phase by ESI and are subsequently fragmented in the mass spectrometer, yielding the molecular masses of both the protein and the fragment ions. If a sufficient number of informative fragment ions are observed, this analysis can provide a complete description of the primary structure of the protein and reveal all of its modifications, as well as any correlations that exist between these modifications. Although the molecular masses of intact proteins have been successfully measured by MALDI– and ESI–mass spectrometry for some time (*3*), it has proved difficult to produce extensive gas-phase fragmentation of intact protein ions, especially from large proteins.

Han *et al.* now demonstrate that they can obtain highly informative fragmentation for proteins with molecular masses extending to more than 200 kD. The authors achieve this remarkable feat by pumping relatively large amounts of energy into the ionized protein throughout the ion injection and collisional dissociation steps, apparently maintaining the protein in an unfolded and conformationally uncollapsed state. In so doing, they considerably improve the prospects for the top-down approach.

Together with the recent introduction of two other highly effective methods for fragmenting large peptides and proteins—electron capture dissociation (*6*) and electron transfer dissociation (*7*)—this critical fragmentation component of the top-down approach now appears within reach. However, other formidable challenges remain to be overcome before the top-down approach can be considered truly robust for proteomics studies, rather than a technique for studying single purified proteins.

One major challenge is the need to separate small quantities of complex mixtures of proteins prior to mass spectrometric fragmentation. The distinctly different physico-chemical properties of different proteins make them difficult to handle as mixtures without incurring overwhelming losses of certain components or rendering the proteins incompatible with ESI-mass spectrometry. This problem has been successfully addressed with sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE), because the association of the detergent SDS with proteins tends to nullify their individual properties. Unfortunately, the presence of ionic detergents, such as SDS, is not compatible with ESI, and this option is therefore not open to top-down proteomic studies. Other possibilities that are compatible with ESI–mass spectrometry include chromatography in agents that keep a wide range of proteins in solution (*8*), separations within the mass spectrometer based on mass (*9*) or ion mobility (*10*), or combinations of these methods.

Equally challenging is the need to separate slightly different forms of the same protein that differ as a result of modifications and in vivo proteolytic processing. Sensitivity is also a major challenge, because effective fragmentation of a high-molecular-mass protein implies that the protein will break up in a very large number of different ways. Thus, the intensity of any given fragment will be weak compared to that from small low-molecular-mass peptides.

Despite these challenges, it seems likely that the bottom-up and top-down approaches will continue to coevolve. Perhaps they will initially meet halfway as a hybrid approach, in which large fragments or whole domains of proteins are analyzed intact. Ultimately, developments such as those described by Han *et al.* should allow us to analyze and describe in detail the complete primary structures of proteins on a proteomic scale.

References and Notes

1. R. Aebersold, M. Mann, *Nature* **422**, 198 (2003).
2. X. Han, M. Jin, K. Breuker, F. W. McLafferty, *Science* **314**, 109 (2006).
3. B. T. Chait, S. B. H. Kent, *Science* **257**, 1885 (1992).
4. A fragmentation ladder is a series of peptide fragments with a common terminus, but differing in length on the opposite side of the peptide.
5. Alternative splicing of a pre-mRNA that is transcribed from one gene can result in the production of different mature mRNA molecules and ultimately in the translation of related but different proteins, i.e., alternative splice variants.
6. R. A. Zubarev *et al.*, *Anal. Chem.* **72**, 563 (2000).
7. J. J. Coon *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **102**, 9463 (2005).
8. R. W. Wozniak, G. Blobel, M. P. Rout, *J. Cell Biol.* **125**, 31 (1994).
9. H. Hernandez *et al.*, *EMBO Rep.* **7**, 605 (2006).
10. R. A. Sowell *et al.*, *J. Am. Soc. Mass Spectrom.* **15**, 1341 (2004).

10.1126/science.1133987

EVOLUTION

Fossil Record Reveals Tropics as Cradle and Museum

Charles R. Marshall

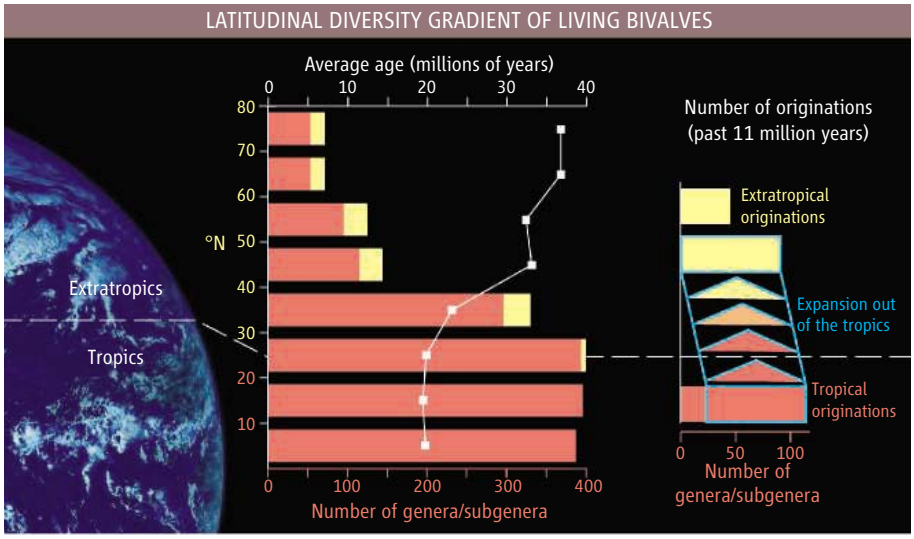
Over the past 11 million years, most bivalves that originated in the tropics expanded their ranges out of the tropics, where they now dominate the living extratropical fauna.

Most groups of organisms show a pronounced decrease in biodiversity from the tropics to the poles. Understanding this long-recognized latitudinal biodiversity gradient requires unraveling the evolutionary dynamics behind it. Typically, work has centered on whether the tropics have unusually high origination rates (in which case they are a cradle of biodiversity), or unusually low extinction rates (in which case they represent a museum of biodiversity). On page 102 of this issue, Jablonski *et al.* (*1*) add a new wrinkle to understanding the evolutionary dynamics of latitudinal diversity gradients by showing that much of the diversity of bivalves outside of the tropics is driven by the expansion of the geographic ranges of species that originated in the tropics. Thus, they argue that the tropics are both a cradle of biodiversity and a museum.

Most studies ignore the possible role of migration in latitudinal diversity gradients. Jablonski *et al*. report the first comprehensive analysis of the fossil record to document the patterns of origination, extinction, and migration. With a meticulously standardized taxonomy, they analyzed the fossil record of 163 genera and subgenera of bivalve mollusks that originated since the beginning of the late Miocene, 11 million years ago.

However, using the fossil record is not straightforward. Jablonski *et al.* had to overcome the relatively poor fossil record of the tropics. The lack of outcrop, the deep weathering of tropical rocks, and the dearth of research effort in the tropics have led to the recovery of, at the very least, 25 times as many bivalve fossils from the extratropics as from the tropics [see note 43 in (*1*)]. Thus, even if a

The author is in the Departments of Organismic and Evolutionary Biology and Earth and Planetary Sciences, Harvard University, 26 Oxford Street Cambridge, MA 02138, USA. E-mail: cmarshal@oeb.harvard.edu



Diversity dynamics. (Left) Cosmopolitan and tropical endemic genera and subgenera (red) dominate the latitudinal diversity gradient of living bivalves [Northern Hemisphere shown only (*18*)]. There are relatively few extratropical endemics (beige). In addition, the age of the living genera (white squares) increases toward the pole (*18*), which suggests a higher extinction rate in the tropics. **(Right)** Analysis of the global fossil record (*1*) shows that most of the genera and subgenera that originated in the tropics over the past 11 million years have expanded their range out of the tropics—that is, the tropics are both the cradle and museum of extratropical diversity.

genus originated in the tropics and then expanded into temperate latitudes, the fossil record would most likely record the opposite signal—an extratropical origin, followed by a range expansion into the tropics. Jablonski *et al.* work around this problem by using genera from only the best-preserved families, those that have at least 75% of their living genera represented in the fossil record. Using this subset of taxa, they show that ~80% of genera that originated in the tropics later expanded into the extratropics (see the figure). This represents so many taxa that only about a third of extratropical genera actually had their origins there. Strikingly, there are virtually no cases of the reverse scenario—extratropical origins followed by range expansions into the tropics.

The recognition of this expansion out of the tropics has led Jablonski *et al.*, via a new route, to corroborate an emerging conclusion (*2*–*4*): that the tropics are both a museum and a cradle of biodiversity. From an extratropical perspective, the tropics (at least for bivalves over the past 11 million years) are a cradle, given that so many genera found in the extratropics have their origins in the tropics, and a museum, given that the earliest history of much of the extratropical fauna is tropical.

Importantly, the exact meanings of "cradle" and "museum" depend on when the terms were used historically, and on the data being analyzed. For example, in the first clear discussion of the tropics as a cradle and/or museum, Stebbins (*5*) argued that the tropics were a museum because, in his view, the tropical taxa were primitive in nature. In the modern language of cladistic systematics, where groups are identified on the basis of evolutionary innovations rather than overall similarity, this translates into the claim that the tropics are a museum because they have a disproportionate number of plesiomorphic taxa (those that retain primitive characteristics while lacking evolutionarily derived characteristics).

In contrast, McKenna and Farrell (*4*), using a temporally calibrated molecular phylogeny, identified the tropics as a museum given their finding of an accelerated rate of diversification early in their leaf beetles' history. More typically, the tropics may be characterized as a museum if they have low extinction rates (*5*–*8*) [which is equivalent to Stebbins's (*5*) notion of a museum if the low extinction rates translate into the persistence of plesiomorphic taxa], and as a cradle (*4*) if they have high origination rates (*9*–*15*). Finally, whether a region is a cradle or a museum may depend on the taxonomic level of analysis. For example, if a clade originated extratropically, migrated to the tropics and radiated there, and then if some of those species migrated back into the extratropics, then the extratropics would be the cradle at a higher taxonomic level, whereas the tropics would be the cradle at a lower taxonomic level.

For the bivalves, the tropics' status as museum may depend on the criterion used for identifying museums. For example, Jablonski *et al.* show that the tropics are a museum in the sense that most extratropical taxa have tropical origins. But they also show that for the living genera, the extratropical ones are typically older. This suggests that the extratropics might have lower per-lineage extinction rates. If true, then the tropics would not be a museum according to the extinction rate criterion. To determine which geographic region is a museum by the extinction rate criterion, we need to know the relative extinction rates of tropical and extratropical endemics. Jablonski *et al.* provide an estimate of the number of tropical endemic extinctions, but are unable to disentangle the extinction rate for the extratropical endemics from that for the cosmopolitan taxa (those that are found both tropically and extratropically).

Jablonski *et al.* conclude by arguing that a major extinction in the tropics would have a major effect on the extratropics as well. This raises interesting questions about the effect of tropical invasions in the extratropics. If there were no tropical invasions, would the diversity in the extratropics be greatly reduced or would there simply be a greater number of extratropical originations? If the latter were the case, what is it about the tropical invaders that suppresses the origination of new genera in the extratropics? Perhaps a way of teasing apart this issue is to compare terrestrial systems, in which migrations out of the tropics appear quite limited (*2*, *16*, *17*), with marine systems, in which expansion out of the tropics may be pervasive.

References and Notes

1. D. Jablonski, K. Roy, J. W. Valentine, *Science* **314**, 102 (2006).
2. J. J. Wiens, M. J. Donoghue, *Trends Ecol. Evol.* **19**, 639 (2004).
3. B. A. Hawkins, J. A. F. Diniz-Filho, S. A. Soeller, *J. Biogeogr.* **32**, 1035 (2005).
4. D. D. McKenna, B. D. Farrell, *Proc. Natl. Acad. Sci. U.S.A.* **103**, 10947 (2006).
5. G. L. Stebbins, *Flowering Plants: Evolution Above the Species Level* (Belknap, Cambridge, MA, 1974).
6. A. R. Wallace, *Tropical Nature and Other Essays* (Macmillan, London, 1878).
7. A. G. Fischer, *Evolution* **14**, 64 (1960).
8. K. J. Gaston, T. M. Blackburn, *Proc. R. Soc. London B* **263**, 63 (1996).
9. J. Haffer, *Science* **165**, 131 (1969).
10. G. T. Prance, *Acta Amazonica* **3**, 5 (1974).
11. A. H. Gentry, *Ann. Mo. Bot. Gard.* **69**, 557 (1982).
12. J. E. Richardson, R. T. Pennington, T. D. Pennington, P. M. Hollingsworth, *Science* **293**, 2242 (2001).
13. S. T. Malcomber, *Evolution* **56**, 42 (2002).
14. C. Hughes, R. Eastwood, *Proc. Natl. Acad. Sci. U.S.A.* **103**, 10334 (2006).
15. J. T. Weir, *Evolution* **60**, 842 (2006).
16. B. D. Farrell, C. Mitter, D. J. Futuyma, *BioScience* **42**, 34 (1992).
17. R. E. Latham, R. E. Ricklefs, in *Species Diversity in Ecological Communities: Historical and Geographical Perspectives*, R. E. Ricklefs, D. Schluter, Eds. (Univ. of Chicago Press, Chicago, IL, 1993), pp. 294–314.
18. Data from supporting online material in (*1*).

10.1126/science.1133351

PHOTO CREDIT: NASA

CANCER

Cancer Immunotherapy Is More Than a Numbers Game

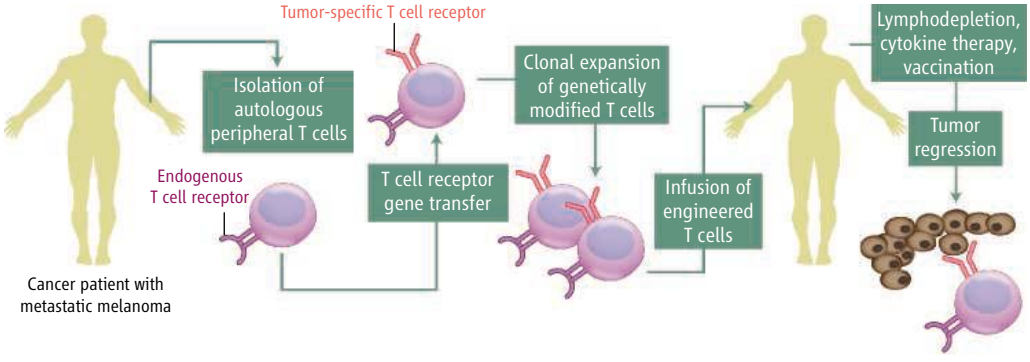
White blood cells that are genetically engineered to destroy human melanoma prove effective for a few patients in a clinical trial. A variety of strategies might increase the success rate.

Rienk Offringa

The concept of manipulating the human immune system to fight cancer has spawned many avenues of potential immunotherapy, from cytokine, drug, and antibody-based treatments to cancer vaccines and gene therapy.

Enhanced online at www.sciencemag.org/cgi/content/full/314/5796/68

Ever since the identification of the first human melanoma molecular marker, or antigen, in the early 1990s (*1*), tumor immunologists have attempted to boost the numbers of tumor-specific T cells in melanoma patients. The study by Morgan *et al*. on page 126 in this issue (*2*) is the first to combine four previously tested cancer treatments—vaccination, genetic engineering, adoptive cell transfer, and cytokine treatment—to tackle human melanoma, or any human cancer for that matter (see the figure). The results are promising, in that two patients who had failed previous therapies showed durable regression of metastatic melanoma. But the success rate of 2 out of 15 patients is disappointing given that all presently available immune weaponry was brought into play. That latter notion raises the question of whether we have now witnessed the maximal potency of cancer immunotherapy. There are many reasons to believe that this is not the case.



An immunotherapeutic regime. Lymphocytes taken from a cancer patient are engineered to express a T cell receptor that recognizes a melanoma-specific molecule. Once expanded, these antitumor lymphocytes are transferred back into the patient to destroy cancer cells.

Increasing the number of desired tumor-targeting T cells can be achieved in two ways: by vaccination with a tumor-specific antigen or by the adoptive transfer of tumor-specific lymphocytes that have been expanded in cell culture. In the case of tumor-associated autoantigens—those molecules also present on one's normal cells—the breadth and quality of one's T cell repertoire may be limited by self-tolerance. This knowledge has prompted researchers to replenish this repertoire through the introduction of genes encoding tumor-specific T cell receptors into primary T lymphocytes (*3*, *4*). Furthermore, various strategies, such as depletion of host lymphocytes and cytokine therapy, have been explored to overcome suppression of the T cell response by immunoregulatory and homeostatic mechanisms. In recent years, the efficacy of these strategies, and combinations thereof, has been tested extensively in preclinical murine tumor models. These animal studies have demonstrated that even though each of the single strategies may affect tumor growth, successful eradication of preexisting malignant tumors requires combination therapies (*5*). Because immunotherapy studies in human cancer patients exploiting single strategies have shown very little clinical efficacy, it was important to test the clinical impact of combination therapies. In this respect, the work by Morgan *et al*. represents a milestone.

As noted by Morgan *et al*., genetic engineering of antitumor T cells can be improved. Vectors used to transfer transgenes can be optimized to prevent silencing of the transgenes over time. Modification of transgene constructs to circumvent mispairing between T cell receptor chains encoded by transgenes and endogenous genes—resulting in decreased expression of functional receptors—also deserves attention. Furthermore, cells constituting the "helper arm" of the T cell response can be brought into action through the expression of T cell receptors with high affinity for tumor antigens. This is expected to enhance clinical efficacy by supporting long-lasting cytotoxic T cell immunity and by increasing the activity of innate immune effector cells. Previous antimelanoma vaccination studies showed tumor escape through loss of antigen expression (*6*, *7*). Therefore, targeting of other antigens in addition to MART-1 (melanoma antigen recognized by T cells–1) is also expected to increase efficacy.

A striking aspect of the data presented by Morgan *et al*. is the high number of successfully engineered (MHC-tetramer positive), MART-1–specific cytotoxic T lymphocytes, detected in the circulation of many of the patients. These T cells constitute up to 1% of the cytotoxic T lymphocyte ($CD8^+$) population, even at prolonged times after adoptive treatment. This suggests that the number of tumor-specific cytotoxic T lymphocytes is not the limiting factor. Because there is no overt correlation between systemic cytotoxic T lymphocyte numbers and clinical efficacy, the absence of therapeutic effects in most of the treated patients seems to be related to the failure of these cells to home in on the tumor and/or to exert their effector function in the context of the tumor microenvironment. Previous clinical antimelanoma vaccination studies have similarly shown that induction of high numbers of tumor-specific cytotoxic T lymphocytes does not necessarily result in antitumor efficacy (*8*, *9*). Interestingly, patients in the present clinical study did not have tumor-infiltrating lymphocytes available for the treatment regime, suggesting that their cancers were relatively nonpermissive to lymphocyte infiltration.

The author is head of the Tumor Immunology Group, Department of Immunohematology and Blood Transfusion, Leiden University Medical Center, Leiden, the Netherlands. E-mail: r.offringa@lumc.nl

CREDIT: P. HUEY/SCIENCE

These functional limitations are also suggested by the apparent absence of vitiligo in the treated patients, which is surprising because antimelanoma efficacy is often accompanied by autoimmune skin depigmentation (*10*–*12*). It is conceivable that prolonged in vitro culturing negatively affects the in vivo homing capacity of cytotoxic T lymphocytes. Our knowledge of the requirements for efficient homing by (human) T cells is incomplete, so at present it is difficult to pinpoint how we could improve this further. Morgan *et al.* also used systemic administration of interleukin-2 (IL-2) to maximize cytotoxic T lymphocyte proliferation and function. However, toxicity limits the use of this cytokine in human subjects, and its impact on T cell immunity has both positive and negative aspects. Other cytokines, such as IL-15, may be more suitable for enhancing the performance of adoptively transferred cytotoxic T lymphocytes (*13*). Alternative tools for unleashing cytotoxic T lymphocyte function include antibodies that block the immune-regulatory effects of the T cell molecules cytotoxic T lymphocyte antigen–4 and PD1 (*14*).

The T cell immune response could likely also be improved through better vaccination strategies. In the Morgan *et al*. study, patients were repeatedly vaccinated with a synthetic peptide representing a minimal MART-1 epitope. But preclinical studies have shown that repeated injection of such minimal peptide epitopes can result in T cell tolerization instead of activation. Minimal epitopes can be exogenously loaded onto all antigen-presenting cells, many of which do not express the desired repertoire of T cell stimulatory signals, the lack of which is known to cause tolerance. This can be circumvented by providing larger antigens that require uptake and processing by dendritic cells, the most potent antigen-presenting cells for T cell activation (*15*).

The outcome described by Morgan *et al*. may not be perfect, but modifications of the treatment regime to increase clinical efficacy can readily be envisioned. Notably, the patients enrolled in the present study suffered from end-stage disease, exhibiting progressive metastatic melanoma, and were refractory to prior therapy with IL-2. Therefore, the best opportunity for improving treatment outcome probably lies in immune intervention in patients with less advanced disease. Cancer immunotherapy remains a great hope, and the work by Morgan *et al.* provides good reason to be encouraged.

References

1. P. Van der Bruggen, *et al.*, *Science* **254**, 1643 (1991).
2. R. A. Morgan *et al*, *Science* **314**, 126 (2006); published online 31 August 2006 (10.1126/science.1129003).
3. T. M. Clay *et al.*, *J. Immunol.* **163**, 507 (1999).
4. T. N. Schumacher *et al.*, *Nat. Rev. Immunol.* **2**, 512 (2002).
5. C. Wrzesinski, N. P. Restifo, *Curr. Opin. Immunol.* **17**, 195 (2005).
6. E. Jager *et al.*, *Int. J. Cancer* **66**, 470 (1996).
7. A. Riker *et al.*, *Surgery* **126**, 112 (1999).
8. S. A. Rosenberg *et al.*, *J. Immunol.* **175**, 6169 (2005).
9. V. Appay *et al.*, *J. Immunol.* **177**, 1670 (2006).
10. Y. Kawakai, S. A. Rosenberg, *Int. Rev. Immunol.* **14**, 173 (1997).
11. W. W. Overwijk *et al.*, *J. Exp. Med.* **198**, 569 (2003).
12. N. Gogas *et al.*, *N. Engl. J. Med.* **354**, 709 (2006).
13. T. A. Waldmann, *Nat. Rev. Immunol.* **6**, 595 (2006).
14. A. J. Korman, K. S. Peggs, J. P. Allison, *Adv. Immunol.* **90**, 297 (2006).
15. C. J. Melief *et al.*, *Immunol. Rev.* **188**, 177 (2002).

10.1126/science.1133893

CHEMISTRY

Boron Goes On the Attack

A newly synthesized boron compound may serve as a nucleophile in a wide range of chemical syntheses.

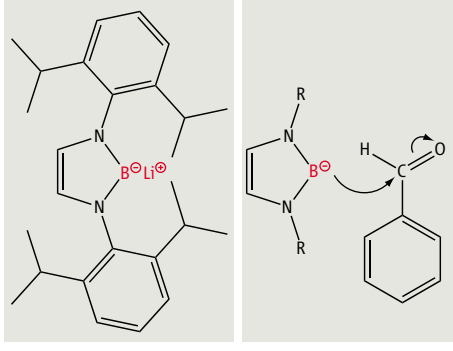
Todd B. Marder

Boron compounds are widely used in synthetic chemistry, but almost all of them are electrophiles (electron acceptors); the few reactions in which boron behaves as a nucleophile (electron donor) are generally catalyzed by a metal. This situation is about to change. On page 113 of this issue, Segawa *et al*. (*1*) report the synthesis and characterization of a boron compound in which the boron center is nucleophilic. This compound may find application in many different areas of synthetic chemistry.

Synthetic chemistry is dominated by two types of reactions: nucleophilic attack and electrophilic attack. Nucleophiles (Lewis bases) possess a filled molecular orbital (an accessible electron pair), which can be stabilized by bonding to an empty orbital on another molecule, causing them to "attack" the other molecule. In contrast, electrophiles (Lewis acids) possess empty low-energy orbitals, which can accept a pair of electrons from a nucleophile. Nucleophilic attack is used to substitute one group for another in organic synthesis, as well as to synthesize many types of compounds from carbonyl-containing organics such as ketones, aldehydes, and esters. Likewise, many routes for attaching ligands to main-group and transition metals involve nucleophilic attack on the metal by the ligand.

Carbon compounds can be either nucleophilic or electrophilic. Examples of the former are organolithium compounds such as methyl lithium (CH_3Li or MeLi) (*2*). Although there is a degree of covalency (and a complex cluster structure) in methyl lithium, it can nevertheless be considered to be a useful form of the carbanion, CH_3^-. Carbon electrophiles include alkyl halides (RX), electron-deficient, three-coordinate carbonium ions (R_3C^+), which contain six electrons rather than the normal eight, and carbonyl compounds, wherein the carbon is the electrophilic site.

Boron is considerably more electropositive than carbon. Because boron is located immediately to the left of carbon in the periodic table, neutral, three-coordinate boranes are typically more stable than their isoelectronic carbonium ion analogs. Boranes are electrophilic and represent an important class of Lewis acids. For example, the boron center in boranes can bind to the oxygen atom (electron pair donor) in carbonyl compounds such as ketones, making the carbon center more susceptible to nucleophilic attack. The chemistry of boron (at least in its three-coordinate compounds), although rich, exciting, and



Unusual nucleophile. The novel lithium boryl compound of Segawa *et al.* (**left**) and its nucleophilic attack on benzaldehyde (**right**).

The author is in the Department of Chemistry, Durham University, Durham DH1 3LE, UK. E-mail: todd.marder@durham.ac.uk

extremely important in organic synthesis (*3*, *4*), is thus dominated by its electrophilic character, and there are few cases (often metal-promoted) in which the boron atom serves as a nucleophile. For example, transition metal-catalyzed additions of $(RO)_2B–B(OR)_2$ to certain C=C and C=O double bonds (diboration) (*5*) could be viewed as a process in which one boron is transferred as a nucleophile and the other as an electrophile. The metal (such as rhodium or platinum) cleaves the B–B bond, facilitating the sequential transfer of the two boryl units to the two ends of the double bond.

Typically, reduction of $XB(NR_2)_2$ compounds (X = Cl, Br) with sodium or potassium leads to dimer formation, resulting in B–B bonded $(R_2N)_2B–B(NR_2)_2$ compounds, especially when R = Me (*6*). This process is important, because $(Me_2N)_2B–B(NMe_2)_2$ is the precursor to a host of $(RO)_2B–B(OR)_2$ compounds; the latter, which have recently become commercially available in bulk quantities, are used in B–C bond-forming reactions catalyzed by transition metals (*5*, *7*–*9*). However, it has not been possible to isolate the anion $(R_2N)_2B^-$ because of the dimerization that occurs with small R groups.

Segawa *et al*. now report the synthesis and complete characterization, including a single-crystal structure, of a $LiB(NR_2)_2$ compound (see the figure). They show convincingly that the boron center is nucleophilic.

Using the bulky RNCH=CHNR (where R=2,6–$^{i}Pr_2C_6H_4$) group on boron, the authors managed to reduce the (RNCH=CHNR)BBr compound to the Li salt of its anion, (RNCH=CHNR)BLi. The large substituents on nitrogen prevent the close approach to a second boron center, which would lead to dimerization during the reduction process. Segawa *et al.* show that this novel compound, which is isoelectronic with the analogous carbon compounds known as N-heterocyclic carbenes that have been made popular by Arduengo *et al.* (*10*), serves as a boron nucleophile, because it can be protonated with H_2O or methylated with a source of Me^+. In addition, the boron attacks the carbon atom in benzaldehyde to produce the corresponding α-borylbenzyl alcohol.

The work opens the door to new pathways in boron chemistry that will have a substantial impact on both organic and inorganic synthesis. For example, one might expect this compound to be used in a host of other nucleophilic additions to organics, including conjugate additions to α,β-unsaturated carbonyls and related compounds, which would provide an alternative to metal-catalyzed diborations of such substrates (*5*). The potential also exists for direct reactions of the new lithium boryl compound with very electrophilic aromatics such as hexafluorobenzene. It remains to be seen whether the products of such reactions can be hydrolyzed to the corresponding boronic acids $[R–B(OH)_2]$, which would be useful reagents in Pd- or Cu-catalyzed cross-coupling processes resulting in C–C, C–O, or C–N bond formation, in addition to other synthetic organic reactions. On the inorganic side, the new boron nucleophile should react with a wide variety of metal halide complexes to give the respective metal-boryl complexes (*11*–*14*), $L_xM–B(RNCH=CHNR)_y$. These complexes would be of interest both fundamentally and because of their potential reactivity with organic substrates.

This is a very exciting time for boron chemistry. The report of Segawa *et al.* opens up an entirely new approach to synthetic inorganic and organic boron chemistry, at a time when both areas are enjoying enormous attention and when their applications abound (*15*).

References and Notes

1. Y. Segawa, M. Yamashita, K. Nozaki, *Science* **314**, 113 (2006).
2. M. J. Lusch, W. V. Phillips, R. F. Sieloff, G. S. Nomura, H. O. House, *Organic Syntheses*, **Coll. 7**, 346 (1990).
3. H. C. Brown, *Boranes in Organic Chemistry* (Cornell Univ. Press, Ithaca, 1972).
4. M. Zaidlewicz, H. C. Brown, *Organic Syntheses via Boranes, Volume 2, Recent Developments* (Aldrich Chemical Co., Milwaukee, 2001).
5. N. J. Bell *et al.*, *Chem. Commun.* 1854 (2004).
6. R. J. Brotherton, A. L. McCloskey, L. L. Petterson, H. Steinberg, *J. Am. Chem. Soc.* **82**, 6242 (1960).
7. T. Ishiyama, N. Miyaura, *J. Organometal. Chem.* **611**, 392 (2000).
8. T. B. Marder, in *Product Subclass 3: Diborane(4) Compounds, Science of Synthesis vol. 6*, D. E. Kaufmann, Ed. (Georg Thieme, Stuttgart-New York, 2005), pp. 117–137.
9. T. B. Marder, N. C. Norman, *Top. Catal.* **5**, 63 (1998).
10. A. J. Arduengo III, R. Krafczyk, R. Schmutzler, *Tetrahedron* **55**, 14523 (1999).
11. G. J. Irvine *et al.*, *Chem. Rev.* **98**, 2685 (1998).
12. M. R. Smith III, *Prog. Inorg. Chem.* **48**, 505 (1999).
13. H. Braunschweig, C. Kollann, D. Rais, *Angew. Chem. Int. Ed.* **45**, 5254 (2006).
14. S. Aldridge, D. L. Coombs, *Coord. Chem. Rev.* **248**, 535 (2004).
15. H. Braunschweig, D. Rais, *Angew. Chem. Int. Ed.* **44**, 7826 (2005).

10.1126/science.1133326

ASTRONOMY

Galactic Prominences on the Rise

The same process that creates arches and loops of plasma on the Sun's surface may be the cause of huge molecular loops found in the center of the Milky Way.

Mark Morris

The central region of our Galaxy contains a massive concentration of interstellar molecular gas that has been surveyed many times. The most often used probe of this gas is emission from the most abundant molecule that can easily be observed, carbon monoxide. On page 106 of this issue, Fukui *et al*. (*1*) extend earlier surveys of CO to a substantially larger region than had previously been mapped. The initial results reveal surprising new structures: giant molecular loops located a few thousand light-years from the galactic center and extending almost a thousand light-years above the galactic plane (*2*).

Interstellar gas in our Galaxy—or in any equilibrated and undisturbed spiral galaxy—resides in a relatively thin rotating disk. It is confined to this disk by the gravitational force of the stars, which are at their densest in the central plane of the galactic disk. The thickness of the gas disk is determined by the degree to which it has been stirred by supernovae and galactic shocks (both of which generate large-scale turbulence) and by the Galaxy's magnetic field, which prevents the gas from collapsing into a much thinner layer. The magnetic field in the disk of the Galaxy is predominantly parallel to the disk, supporting the gas against the largely "vertical" gravitational field (here, "vertical" means perpendicular to the disk).

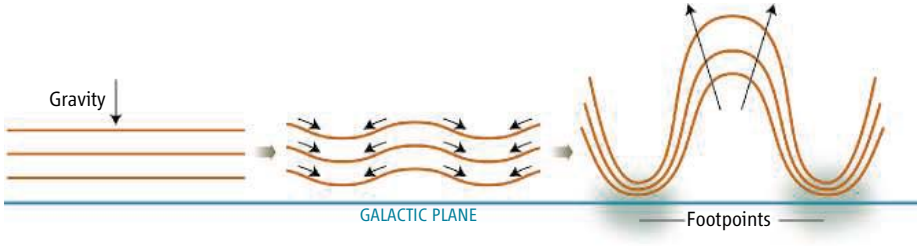
In 1966, however, Parker (*3*) pointed out that this configuration is not stable: Any vertical indentation in the magnetic field on a sufficiently large scale will provide a "pool" into which interstellar gas will sink as it slides down the tipped field lines in response to gravity (see the figure). As gas is unloaded from the higher lying sections of the field lines, the field becomes increasingly buoyant and thus rises there (*4*). At the same time, as the pooled section gathers mass, it will sink toward the galactic midplane, deforming the field more, accelerating the flow of gas into

The author is in the Department of Physics and Astronomy, University of California, Los Angeles, CA 90095, USA. E-mail: morris@astro.ucla.edu

it. As the resulting gas concentrations become denser, the gas cools relatively quickly, creating dense molecular clouds or cloud complexes via the Jeans instability (*5*). Because star formation in our Galaxy takes place primarily in molecular clouds, this combined Parker-Jeans instability might play an important role in fostering much of the star formation.

The Parker instability is well established among theorists and operates in the Sun, playing a role in the generation of arches, loops, and prominences, and possibly in the production of sunspots. Yet it has not been definitively observed to be operating on galactic scales for want of a good diagnostic (*6*). Fukui and his collaborators argue that the giant molecular loops they observe have resulted from the Parker instability. The radial velocity field that they infer from the Doppler shifts of the CO emission indicates that these are indeed loops, rather than shells, and that there is a flow of material along the loops, as is anticipated by the theory (*7*). Also, the size of the galactic loops is in the range expected for the Parker instability. This is an exciting development, raising the possibility of directly confirming this phenomenon on a galactic scale—a scale larger by 12 orders of magnitude than the magnetic loops seen on the Sun.

Two challenges are presented by this interpretation, however, both of which might be met with more observational work. The first is that there is so far no evidence of a magnetic field in the loops, which could be resolved by measurement of polarized emission caused by magnetic alignment of dust grains (*8*). The galactic center is indeed known to have a strong magnetic field (*9*), although it has not yet been measured as far out as the loops are located. Furthermore, the field within 300 or 400 light-years of the galactic center is vertical, which would not support the Parker instability. Thus, the presence of the molecular loops farther out—if indeed they result from a Parker mechanism—necessitates that the field orientation shift to the horizontal configuration characteristic of the rest of the galactic disk somewhere between 400 and 1000 light-years from the center. Also, if the strength of the galactic magnetic field at the loops remains high relative to the field farther out in the disk, then we can perhaps understand why this is the place where they were discovered: Stronger magnetic loops are more buoyant.



Loop formation. Successive stages of the Parker instability occurring above the galactic plane. (**Left**) An idealized set of magnetic field lines oriented parallel to the midplane of a disk-shaped galaxy. The real galactic magnetic field is subject to considerable distortion about the mean field shown. (**Middle**) Any vertical perturbation to the field lines begins the instability and causes gas to slide down the tilted field lines. (**Right**) As the instability develops, large magnetic loops form above the galactic plane, and the gas concentrated at the base of the magnetic loops may form molecular clouds. Unless the tops of the loops are weighed down by dense gas, they will keep expanding into the halo of the galaxy.

The other challenge is to explain why magnetic loops resulting from the Parker instability are outlined by molecular gas. Descriptions of the Parker instability have assumed only a diffuse, low-density atomic gas, because that is what is typically present in the interstellar medium far above the galactic plane where the instability manifests itself. Molecular clouds had previously been thought to form by the Parker-Jeans instability only at the "footpoints" of the loops, where they join the gas layer in the galactic plane. Molecular gas may have been levitated from near the galactic gas layer by the buoyancy of the magnetic field, but if the gas is flowing down the sides of the loop along the magnetic field lines, why is there so much gas left high above the galactic plane? One possible answer to this question is that the rising portion of the magnetic loop has shocked and compressed the relatively rarified atomic gas in front of it, leading to rapid cooling and ultimately to a phase transition from atomic to molecular gas. If so, then the molecular gas defining the loops is constantly being replenished.

Deformation of the horizontal magnetic field by the Parker instability generates vertical magnetic field components out of a horizontal field; thus, it is interesting to contemplate how the newly discovered loops might relate to the strong vertical field closer to the galactic center. The magnetic field in the two vertical legs (or "flux tubes") of a rising loop should persist with opposite polarity (i.e., opposite field direction), even after the top of the loop has completely broken out of the galactic gas layer and expanded into the galactic halo. If those magnetic legs are then transported toward the center of the Galaxy by the inexorable inward migration of their footpoints (*10*), then all the vertical magnetic flux tubes produced by this process will pile up there. As they are brought into contact, the flux tubes of opposite polarity can interact with each other, through a process called field line reconnection, and deposit energy along their boundary. This provides a possible explanation for another phenomenon that radio astronomers have studied for more than 20 years: a population of vertical, radio-emitting, magnetized filaments in the inner few hundred light-years of the Galaxy (*11*). Because it is these filaments that initially led me and other astronomers to conclude that there exists a vertical magnetic field at the galactic center, it seems worthwhile to now reconsider the whole picture from a broader perspective. The loops reported by Fukui *et al.* may well give us a handle on this complex process.

References and Notes

1. Y. Fukui *et al.*, *Science* **314**, 106 (2006).
2. For comparison, our distance from the galactic center is ~25,000 light-years.
3. E. N. Parker, *Astrophys. J.* **145**, 811 (1966).
4. The buoyancy of the magnetic loop is augmented by the pressure of the "gas" of galactic cosmic rays. These essentially light-speed particles inflate the region encompassed by the loop.
5. B. G. Elmegreen, *Astrophys. J.* **253**, 634 (1982).
6. Some relatively nearby magnetic structures lying well above the galactic plane have been interpreted as manifestations of the Parker instability, but these are more readily explained as compressed, magnetized shells lying at the surfaces of large, expanding bubbles in the interstellar medium caused by supernovae and other energy injection processes associated with massive star formation.
7. Without access to information about the velocity perpendicular to our line of sight, there is an ambiguity about whether this flow goes up or down, but the expectation from the Parker instability is that it goes toward the galactic plane.
8. G. Novak *et al.*, *Astrophys. J.* **583**, L83 (2003).
9. M. Morris, E. Serabyn, *Annu. Rev. Astron. Astrophys.* **34**, 645 (1996).
10. The footpoints are strongly tied to the gas in the galactic plane, and they will therefore follow that gas as it slowly migrates inward toward the galactic center by losing angular momentum through a variety of processes, one of which is the torque exerted by these magnetic columns that the gas is dragging along.
11. F. Yusef-Zadeh, J. W. Hewitt, W. Cotton, *Astrophys. J. Suppl.* **155**, 421 (2004).
12. I thank F. Coroniti for a thought-provoking discussion.

10.1126/science.1133500

EPPENDORF 2006 WINNER

A Dedicated System for Processing Faces

In the regions of the brain that are most highly activated by faces, single cells make different measurements that are pooled together to characterize the face.

Doris Tsao

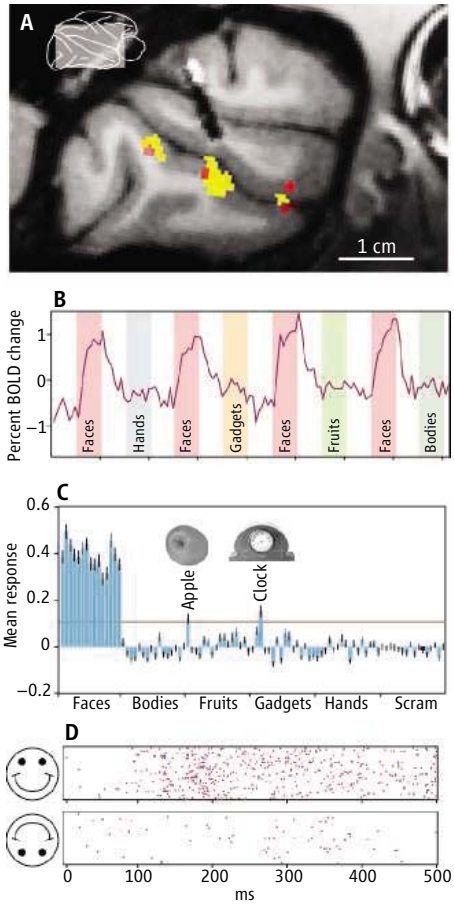
Eppendorf and *Science* are pleased to present the prize-winning essay by Doris Tsao, the 2006 winner of the Eppendorf and *Science* prize for Neurobiology.

If you're planning to rob a bank, there's one thing you must not forget: to cover your face. Otherwise, just a brief glance will allow all the other social animals around you to identify you. What is the neural basis of the extraordinary ability of humans to recognize faces? Localized strokes can selectively destroy face recognition abilities while preserving the ability to recognize other objects ("prosopagnosia") (*1*). Furthermore, functional magnetic resonance imaging (fMRI), a technique that measures blood flow changes induced by brain activation, consistently reveals several discrete brain regions that respond more to faces than to other objects (*2*). One of these regions, the fusiform face area, shows increased blood flow even when subjects merely imagine faces (*3*). These findings suggest that face processing is mediated by specialized modules inside the human brain. Such specialization is surprising since, from introspection, it seems that our recognition of faces flows seamlessly into that of all the other objects in the world.

Are face-selective regions unique to humans? Charles Gross and co-workers studied a large region in the macaque brain known as the temporal lobe and reported in 1981 that this region contains some cells that respond exclusively to faces and not to other visual forms (*4*). This was a remarkable finding: How can a single cell be wired to detect something so complex as a face? The discovery immediately turned fuzzy questions about holistic integration and gnostic units into a concrete research goal: What are face cells detecting, and how are they wired?

One problem, however, stood in the way of understanding these cells: It was difficult to find them. In single-unit recording experiments, one can see only as far as the tip of one's electrode (≤100 μm wide). Several groups that studied face cells reported that they were scattered throughout the temporal lobe, with at most 10 to 20% of the cells in any one region being face-selective (*5*–*7*). Meanwhile, the discovery by fMRI of face-selective regions in humans generated great interest in understanding what is being coded by these regions. One might guess that the fMRI-identified face-selective regions contain lots of face cells. Alternatively, they could contain cells activated by any animate object, or by symmetrical objects, or by the behavioral process of fine scrutiny. Indeed, fMRI evidence was marshaled for several competing theories about face-selective activation.

In order to clarify the link between face cells and fMRI face areas, I performed fMRI experiments in alert monkeys to look for face-selective regions (*8*). Comparing activation to faces versus five other object categories (fruits, bodies, gadgets, hands, and scrambled patterns) across the entire macaque brain, I identified face-selective activation in three discrete regions of the temporal lobe (see the figure, left, panel A). These regions showed a blood oxygen level–dependent (BOLD) response to faces that was stronger than the response to any of the nonface categories by a factor of 7 (see figure, panel B). This suggests that face processing in monkeys is performed by specialized regions, possibly homologous to those found in humans. Furthermore, the arrangement of the face regions along an anterior-posterior axis suggests a hierarchy, given that we know from other studies that complexity in shape selectivity increases from the back to the front of the visual system.



Having found these fMRI face regions in monkeys, I then asked: What is the selectivity of single neurons within an fMRI-identified face patch? I started by recording from single neurons—almost 500 of them—in the middle face patch of two monkeys, and found 97% of the visually responsive neurons to be face-selective (*9*) (see figure, panel C). These cells responded almost 20 times as strongly to faces as to other objects, and many were even suppressed by nonface objects. Up to now, one major difficulty with understanding object recognition has been the problem of determining which object, among an infinite number of possible objects, a single cell in the temporal lobe might be coding. The existence of a region in which all the cells are coding faces goes a long way toward overcoming this difficulty.

What is it about a face that these cells like? Surprisingly, most cells responded to human, monkey, and even highly simplified

Recognizing faces: (**A**) Three patches of face-selective fMRI activation (yellow regions) in the macaque temporal lobe. (**B**) Time course from the face patches. Blood flows to these regions only when the monkey views faces. (**C**) Average response across 182 cells from the middle face patch of one monkey to 96 different images. The first 16 images are faces. (**D**) Responses of a face cell to repeated presentations of an upright and an inverted cartoon face. Each dot represents an action potential.

The author is at the Institute for Brain Research and Center for Advanced Imaging, University of Bremen, D28359 Bremen, Germany. E-mail: doris@nmr.mgh.harvard.edu

cartoon faces (see figure, panel D). In fact, many of the cells showed a weak but significant response to a few particular nonface objects; all of these objects turned out to be round (see figure, panel C). The weak but significant responses to round clocks and fruits in this area, as well as its relatively posterior position within the temporal lobe, indicate that it constitutes an early stage in the form-processing hierarchy. Recording from this area is a bit like peeking into a carpenter's shop and seeing the rough frame before fine chiseling—exactly what one wants for piecing together the basic mechanisms underlying face selectivity.

How do face cells encode specific faces? Early recordings in the middle face patch suggested that face cells distinguish faces on the basis of visual shape (e.g., the cells responded weakly to the round outline in a clock and an apple). To explore shape tuning of these cells quantitatively, I took advantage of their robust response to cartoon faces, which can be easily parameterized. I probed face cells with a cartoon face space consisting of 19 different feature dimensions, each sampled at 11 values; the space thus contained 11^{19} possible different faces. The cartoon dimensions included ones describing the overall facial shape, the shape of individual features (e.g., iris size), and the relationship between features (e.g., intereye distance). Across the population, a vast majority of cells showed strong tuning to at least one cartoon dimension, and no cell was tuned to more than eight dimensions. The two most popular dimensions were face aspect ratio (i.e., Bert versus Ernie) and iris size. Most cells responded best to extreme features such as large irises, Ernie's or Bert's face, etc. These results show that we can understand face cells: Each cell acts as a set of face-specific rulers, measuring faces along multiple distinct dimensions. By combining the measurements of all these little rulers, it should be possible to reconstruct any face (including a bandit's, if not covered well).

My experiments show that the neural machinery for face processing in macaque monkeys consists of a set of discrete brain regions packed with highly dedicated components. This system offers a unique opportunity for exploring high-level form perception. By recording from several large, homogeneous populations of face cells identified through monkey fMRI, we can now understand the process by which the brain synthesizes the percept of a face in terms of underlying single-cell components.

References and Notes

1. J. Bodamer, *Arch. Psychiatr. Nervenkrankh.* **179**, 6 (1947).
2. N. Kanwisher, J. McDermott, M. M. Chun, *J. Neurosci.* **17**, 4302 (1997).
3. K. O'Craven, N. Kanwisher, *J. Cogn. Neurosci.* **12**, 1013 (2000).
4. C. Bruce, R. Desimone, C. G. Gross, *J. Neurophysiol.* **46**, 369 (1981).
5. D. I. Perrett *et al.*, *Hum. Neurobiol.* **3**, 197 (1984).
6. R. Desimone, T. D. Albright, C. G. Gross, C. Bruce, *J. Neurosci.* **4**, 2051 (1984).
7. G. Baylis, E. Rolls, C. Leonard, *J. Neurosci.* **7**, 330 (1987).
8. D. Y. Tsao, W. A. Freiwald, T. A. Knutsen, J. B. Mandeville, R. B. Tootell, *Nat. Neurosci.* **6**, 989 (2003).
9. D. Y. Tsao, W. A. Freiwald, R. B. H. Tootell, M. S. Livingstone, *Science* **311**, 670 (2006).

10. All the experiments here were performed together with Winrich Freiwald. I owe deepest thanks to my adviser Margaret Livingstone and to my father Thomas Tsao.

10.1126/science.1135163

2006 Grand Prize Winner

The author of the prize-winning essay, Doris Tsao, was born in Changzhou, China, and grew up in College Park, Maryland. Dr. Tsao studied biology and mathematics at Caltech, receiving her B.S. in 1996. She moved on to do graduate work in the laboratory of Dr. Margaret Livingstone at Harvard Medical School, where she studied binocular depth perception. While a graduate student, she became interested in monkey fMRI as a way to chart unexplored regions of the brain, and worked together with Roger Tootell to image macaque brain regions involved in depth and face perception. She received her Ph.D. in 2002 but remained as a postdoctoral fellow in Dr. Livingstone's laboratory in order to continue her experiments on face perception. In 2004, she received a Sofia Kovalevskaya Award from the Humboldt Foundation. This award allowed her to set up her own lab at the University of Bremen, Germany. Dr. Tsao's goal is to understand how a sheet of cells 2 mm thick can construct a three-dimensional world and effortlessly recognize the multitude of objects within it. Her laboratory uses a combination of electrophysiology, imaging, psychophysics, and anatomical techniques. Outside the laboratory, she likes to swim, cook, and play the violin.

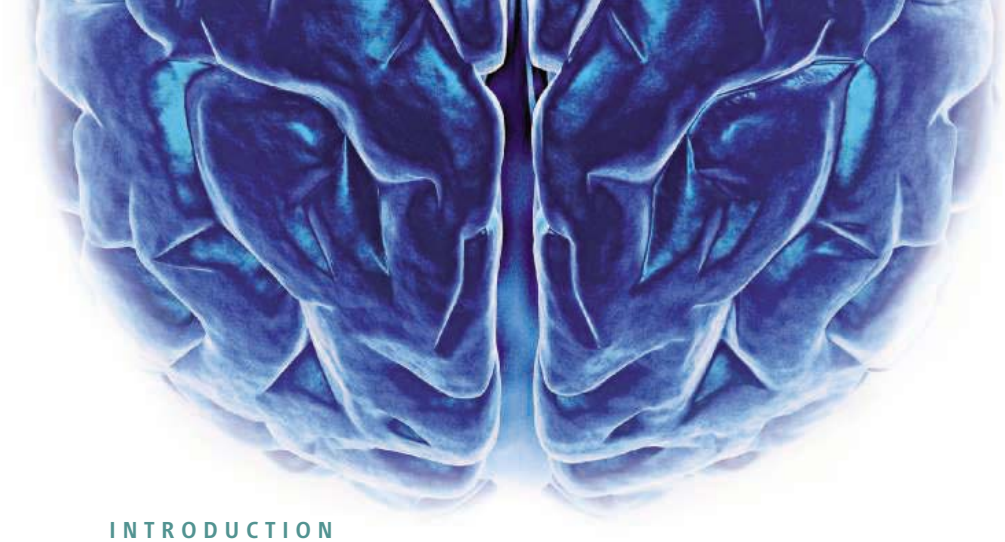
Finalists

Bernardo Sabatini for his essay, "Establishing synaptic independence: How neurons create diffusional barriers." Dr. Sabatini was born and raised in New York. He received his undergraduate degree in biomedical engineering from Harvard College in 1991. He received his M.D. and Ph.D. degrees in 1999 from Harvard Medical School, having completed his thesis work in the laboratory of Dr. Wade Regehr. After graduation, he joined the lab of Dr. Karel Svoboda at Cold Spring Harbor Laboratory as a postdoctoral fellow. In 2001, Dr. Sabatini started his own laboratory in the Department of Neurobiology at Harvard Medical School, which is focused on understanding the processes that regulate the structure and function of synapses and how these processes are perturbed in neurological diseases. His life outside of science is mostly spent trying to keep up with his three sons.

Gábor Tamás for his essay, "Lighting the fire in cortical microcircuits: Exciting role for chandelier cells." Dr. Tamás was born in Dunaújváros, Hungary, and completed undergraduate studies in biology at the University of Szeged, Hungary. As a graduate student he was trained in neuroanatomy and physiology in the group of Peter Somogyi at the University of Oxford, where he investigated the function, number, and location of synapses between neocortical neurons. In 1998, Dr. Tamás returned to Szeged to establish his own laboratory and identified the first intercellular mechanism capable of synchronizing cortical neurons at gamma frequency. His group discovered that the so-called neurogliaform interneuron is capable of eliciting slow, $GABA_B$ receptor–mediated inhibition in the cerebral cortex. Dr. Tamás was a gymnast for 15 years but now gets his exercise from whitewater rafting, skiing, and hiking in the mountains.

For the full text of essays by the finalists and for information about applying for next year's awards, see *Science* Online at www.sciencemag.org/feature/data/prizes/eppendorf/eppenprize.shtml.

CREDITS (LEFT TO RIGHT): WINRICH FREIWALD, ÉVA CSAJBÓK, JENNIFER WILSON



INTRODUCTION

Of Bytes and Brains

COMPUTATIONAL NEUROSCIENCE IS NOW A MATURE FIELD OF RESEARCH. In areas ranging from molecules to the highest brain functions, scientists use mathematical models and computer simulations to study and predict the behavior of the nervous system. Simulations are essential because the present experimental systems are too complex to allow collection of all the data. Modeling has become so powerful these days that there is no longer a one-way flow of scientific information. There is considerable intellectual exchange between modelers and experimentalists. The results produced in the simulation lab often lead to testable predictions and thus challenge other researchers to design new experiments or reanalyze their data as they try to confirm or falsify the hypotheses put forward. For this issue of *Science*, we invited leading computational neuroscientists, each of whom works at a different organizational level, to review the latest attempts of mathematical and computational modeling and to give us an outlook on what the future might hold in store.

Understanding the dynamics and computations of single neurons and their role within larger neural networks is at the center of neuroscience. How do single-cell properties contribute to information processing and, ultimately, behavior? What level of description is required when modeling single neurons? Herz *et al*. (p. 80) review single-cell models, from detailed and reduced compartmental models to point neurons and black-box models and they highlight the merits and corresponding problems.

Single neurons are part of larger networks. Destexhe and Contreras (p. 85) review advances in the computations created by stochastic input in neurons and networks of neurons. They emphasize the importance of irregular activity in neuronal computations.

On a higher processing level, computational neuroscience based on the detailed anatomy and physiology of the human brain can help us understand the complexities of conscious awareness and human intelligence. O'Reilly (p. 91) reviews developments in models, of higher-level cognition. He develops the idea that the prefrontal cortex represents a synthesis between analog and digital forms of computation.

As this special issue's News section demonstrates, computational neuroscience attracts its share of atypical brain researchers. On page 76, Miller describes how Jeff Hawkins, an electrical engineer who invented the PalmPilot, has developed a theory for how the cortex makes predictions. He even founded a small neuroscience institute. And on page 78, Wickelgren looks into the work of Eero Simoncelli, an electrical engineer who seeks to model how the brain's visual system makes sense of the world.

Two Signal Transduction Knowledge Environment (STKE) Reviews concern adaptive and maladaptive consequences of neuronal activity. Lisman and Raghavachari develop a structural model that incorporates seemingly contradictory data to provide a coherent view of long-term potentiation at the hippocampal CA1 synapse. McNamara, Huang, and Leonard hypothesize that activity-dependent increases in Ca^{2+} concentration in dendritic spines are critical to limbic epileptogenesis. In an STKE Perspective on D-serine regulation of *N*-methyl-D-aspartate receptor activity, Wolosker discusses the possibility that D-serine released from neurons and glia may have distinct functions.

– PETER STERN AND JOHN TRAVIS

Modeling the Mind

CONTENTS

See also related Editorial page 17; Books et al. *page 57;* Science*'s STKE and* Science *Careers material on page 11 or at www.sciencemag.org/sciext/compneuro*

Science

NEWS

An Enterprising Approach To Brain Science

Mobile computing pioneer Jeff Hawkins has had a lifelong fascination with brains. Now he's trying to model the human cerebral cortex—and he's created a software company based on his ideas

BOLD IDEAS COME NATURALLY TO JEFF Hawkins. In California's Silicon Valley, Hawkins is well-known as the inventor of the PalmPilot, the first commercially successful handheld computer, and the Treo smartphone. These devices are rarely out of arm's reach for millions of businesspeople, who rely on them to keep track of power lunches and peek at e-mail during meetings. Hawkins, at age 49, could easily retire. Instead, he's on a mission to figure out the brain.

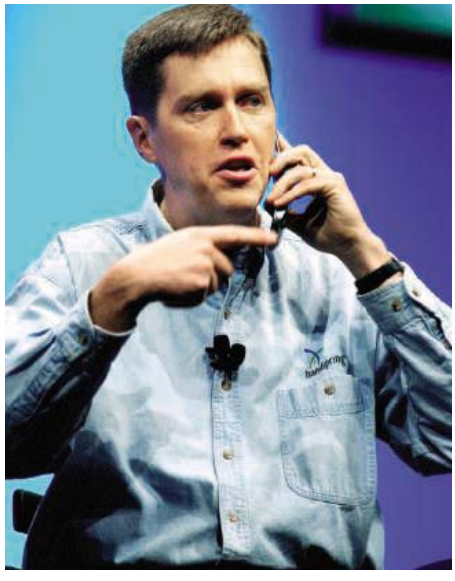
Hawkins has spent a remarkable amount of time thinking about brains, at least for someone who launched a billion-dollar business and values time with his family. Over the past 20 years, he's spent countless hours poring over research papers, sitting in on neuroscience conferences, and hashing out ideas with academic scientists. "Even at Palm, I had an agreement that I could work part-time on brain research," he says. "It was in my contract."

Hawkins's foray into neuroscience is characterized by the same determination and just-do-it attitude that made him a successful entrepreneur. In the past 4 years, he has founded a small neuroscience institute, published a book outlining his theory on the nature of human intelligence, and founded a start-up company to develop computers that work on the same principles. His aggressive approach disconcerts some scientists, who are used to measuring progress one peer-reviewed paper at a time. Yet several prominent neuroscientists say his ideas on the brain are worth taking seriously, and even skeptics say his enthusiasm and entrepreneurial attitude have enlivened the field.

"Jeff is a very interesting and dynamic person," says Michael Merzenich, a neuroscientist at the University of California (UC), San Francisco, who has talked brains with Hawkins over the past 10 years. "He's one of the dozen or so smartest people I've met in my life." Hawkins brings incredible focus and an entrepreneur's sense of urgency to his endeavors, adds Anthony Bell, a theoretical neuroscientist at UC Berkeley who has worked with Hawkins. "He wants a computer program that works like the cortex, and he wants it now," Bell says. "He wants the brain in silicon."

Academic frustration, corporate success

Hawkins may have acquired the impulse for innovation from his father, a consummate inventor whose creations included a 16-sided, 50-ton boat that floated on a cushion of air generated by a vacuum cleaner motor. Growing up on Long Island, Hawkins and his brothers helped build the craft, which they nicknamed the Bubble Monster. Like his dad, Hawkins grew up to be an engineer.



Mobile man. After designing hand-held computers and smartphones for 15 years, Jeff Hawkins is on a quest to understand the brain.

In 1979, fresh out of Cornell University with an undergraduate degree in electrical engineering, Hawkins read a magazine article that he says changed his life. In an essay in *Scientific American*, Francis Crick, whose interests had recently turned from molecular biology to the mysteries of the mind, lamented the lack of a grand unified theory in neuroscience. Scientists have amassed a wealth of details about brain anatomy and physiology, Crick wrote, but still have no working hypothesis of how the whole thing actually works. To Hawkins, this was a call to action.

In 1980, he tried to persuade his employer, computer-chip maker Intel, to let him start a brain research group, but the company declined. The following year, the Massachusetts Institute of Technology (MIT) rejected his application to pursue a Ph.D. in its artificial-intelligence laboratory. Although disappointed, Hawkins resolved to figure out a way to one day pursue his interest in the brain. In 1986, he tried the academic route once more. This time, he was accepted to a graduate program at UC Berkeley.

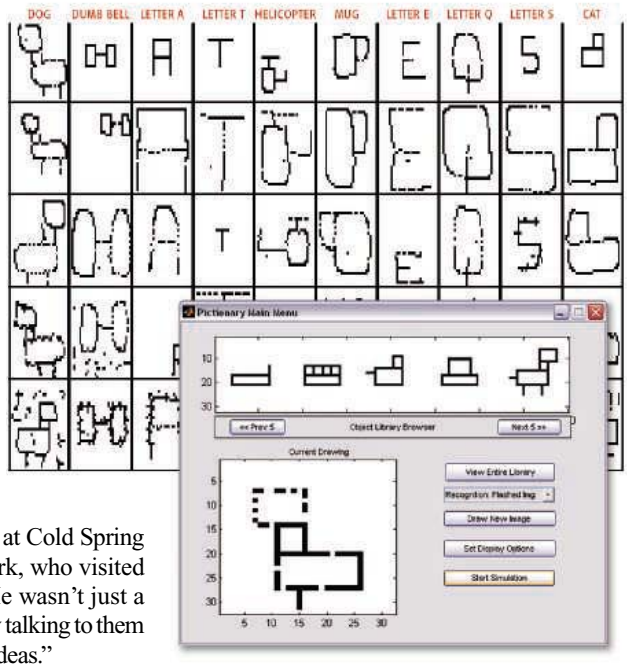
Once there, Hawkins sketched out a theory of how the cerebral cortex—the thin sheet of tissue on the surface of the brain—gives rise to intelligence, and he submitted this as his thesis proposal. "They said, 'This is interesting, but you can't do it,' " he recalls. To get a Ph.D., you need a thesis adviser, and no one at Berkeley was doing theoretical neuroscience back then, he says. Hawkins grew frustrated and impatient. "I was technically a student for 2 years, but by the second year, I was just using the library."

Hawkins returned to the high-tech industry, where he'd gained expertise and a reputation for creativity in designing portable computers. Thanks in part to work he did at Berkeley on neural mechanisms of pattern recognition, he owned a patent on a handwriting-recognition program that allowed computer users to enter data by writing on a screen with an inkless pen. Determined to incorporate this software into handheld computers, Hawkins founded Palm Computing in 1992.

In some ways, the timing couldn't have been worse. In 1993, Apple released a handheld computer, the Newton, and it was a colossal flop. Suddenly, no one wanted to invest in mobile computing, Hawkins says. But he stuck with it. One night in his garage, he carved a mockup of the device he envisioned from a block of wood. As his team worked on the interface, Hawkins tested various configurations of buttons and display windows by sticking printouts onto the model. Silicon Valley lore now has it that he would pull the model out of his pocket during meetings and poke at the "screen" with a sawed-off chopstick, pretending to enter appointments. "I just knew it was going to happen," he says.

Time ultimately proved Hawkins right: Palm has sold more than 34 million devices. In 2002, Hawkins felt the time had come to get back to brains. Scaling down his hours at Palm, he

CREDIT: GETTY



You call that a dog? A Numenta simulation program (*bottom*) recognizes objects even when they're poorly rendered (*top*).

CREDIT: J. HAWKINS

founded (and funded) the Redwood Neuroscience Institute (RNI) in offices above a popular café in Menlo Park, California. The idea, he says, was to bring together scientists interested in creating computational models of the cerebral cortex. The group ultimately consisted of five principal investigators in addition to Hawkins, plus a handful of postdocs. "He collected a great group of very creative people," says Anthony Zador, a computational neuroscientist at Cold Spring Harbor Laboratory in New York, who visited the institute several times. "He wasn't just a manager; he was there every day talking to them about their ideas and about his ideas."

The institute defied categorization. "The atmosphere was a little bit between a start-up, and a think tank, and a research institute," says Fritz Sommer, a computational neuroscientist who left a faculty position at the University of Ulm in Germany to join RNI. "I was kind of an old-school guy, raised in the old German academic tradition, so for me this was something much more inspiring." In the absence of teaching obligations and grant proposals, freeform interactions flourished among the scientists who worked at RNI, says Sommer—and extended to their guests. A visiting speaker was liable to endure a barrage of queries throughout the presentation, making it less like a formal lecture and more like a lively conversation—one that often continued for hours at the café downstairs.

At one meeting, co-sponsored with the American Institute of Mathematics, RNI brought together anatomists, physiologists, and theoreticians who were all studying the cortex. Hawkins asked each group to go off on its own and come up with a list of things the other groups could do that would aid them in their own work. "The scientists were entirely puzzled because no one had ever asked them to do that," says Sommer. "That blew my mind," Hawkins says. "In industry, you're always trying to get help from wherever you can."

Making predictions

Hawkins spent much of his time at RNI fleshing out the ideas on the cerebral cortex he'd conceived at UC Berkeley decades earlier. In 2004, he published them in a book co-written with *New York Times* science writer Sandra Blakeslee. In *On Intelligence*, Hawkins argues that the nature of intelligence—and the primary function of the cortex—is predicting the future by remembering what has happened in the past.

A key feature of Hawkins's argument is the idea of a common cortical algorithm, proposed in the late 1970s by Vernon Mountcastle, a neurobiologist at Johns Hopkins University in Baltimore, Maryland. Because anatomists had found that the type and arrangement of cells in any tiny patch of cortex is very nearly the same, Mountcastle proposed that every patch of cortex performs the same basic operation. What makes one swath of cortex a "visual" region or a "language" region is the kind of information it receives, not what it does with that information. "In my mind, this is one of the most fundamental breakthroughs in neuroscience," Hawkins says.

He is convinced that the common cortical algorithm performs predictions. In the book, he argues that the anatomy of cortex is well-suited for prediction and describes how circuits of cortical neurons arranged in a hierarchy—in which higher levels constantly feed information back to lower levels—can compare an incoming sequence of patterns (such as a string of spoken words) with previously experienced sequences ("Fourscore and seven years") to predict what's next ("ago"). This memory-prediction framework has evolved to take advantage of the spatial and temporal structure in our surroundings, Hawkins says, which helps explain why brains easily do certain tasks that give computers fits. "There's no machine in the world that you can show a picture of something and have it tell you whether it's a dog or a cat or gorilla," he says, but a person can do this in a fraction of a second.

"His ideas … provide a plausible conceptual framework for a lot of different kinds of data," says Mriganka Sur, a neurobiologist at MIT who studies the cortex. Yet some theoretical neuroscientists, none of whom would agree to be named, grumble that Hawkins's book merely rehashes other people's ideas and that his model isn't concrete enough to suggest experiments to test it. Although there's some truth to that, says Sommer, Hawkins has tied together several existing concepts in an interesting way: "He makes connections and sees the bigger picture that people who are doing research on a particular system of the brain often lose."

Hawkins now believes that the best way to spur interest in his cortical theory is to use it to develop technology. "People work harder and get things done faster if they can see a profit motive," he says. Last year, he founded Numenta, a for-profit company, and handed off RNI to UC Berkeley, along with an endowment to cover much of its operating expenses. Now called the Redwood Center for Theoretical Neuroscience, it's part of the Helen Wills Neuroscience Institute.

At Numenta, Hawkins has worked with Dileep George, a former Stanford University electrical engineering grad student, to develop software based on the memory-prediction theory. Hawkins expects the software to be ready for public release next year. In the more distant future, he envisions intelligent computers that will tackle all sorts of problems. In *On Intelligence*, he imagines feeding real-time data from a global network of sensors into a "weather brain" that tracks weather systems in the same way the brain identifies objects and predicts how they will move across the field of vision. By applying humanlike intelligence to vast amounts of data, such a system could identify previously unknown weather phenomena (along the lines of the El Niño cycle) and make more accurate forecasts, Hawkins speculates. Intelligent systems using Numenta's software might also monitor power grids to help guard against blackouts, or monitor sensors on an automobile and alert the driver to dangerous situations.

It's far too early to know whether Hawkins's vision will pan out. But regardless of whether he succeeds, Hawkins has helped galvanize the theoretical neuroscience field, Zador says: "The fact that he's setting these wildly ambitious goals and has set about achieving them is actually quite refreshing."

–GREG MILLER

NEWS

Vision's Grand Theorist

Eero Simoncelli has an eye for mathematical truths that explain human vision—and he's adept at translating that knowledge into practical tools such as image-compression techniques

A great divide traditionally separates theory from experiment in neuroscience. Theorists typically deal in idealized mathematical abstractions far removed from nitty-gritty physiological data. Experimental neuroscientists often view such musings with disdain, considering them irrelevant or too mathematically dense to be of any use.

Eero Simoncelli, a Howard Hughes Medical Institute vision researcher at New York University (NYU), is one of a small but growing cadre of computational neuroscientists bridging this divide. Forty years after researchers revealed the cellular fundamentals of vision, how the electrical signals delivered by the eye's rods and cones assemble into full-scale visual perceptions remains largely an enigma. To sharpen the picture, Simoncelli is working to make neuroscience more like physics, a field in which theory and experiment more easily blend. Just as physicists replaced loose, qualitative descriptions of the physical world with mathematically precise language, Simoncelli aims to devise fundamental equations of vision. "I'm working to encapsulate the conceptual principles used by the brain in precise mathematical terms," he says.

Simoncelli's analyses have already solved several long-standing mysteries in visual science: for example, how the brain assembles a moving picture of the world and why humans drive too quickly in the fog. He's also helped explain how evolution may have sculpted the brain to respond ideally to the visual environment on Earth. On a more practical side, Simoncelli has developed novel methods for image compression and for cleaning up visual noise, such as TV snow. "Eero can hang out with the people who make JPEGs look better or compress info onto DVD," says NYU neuroscientist Anthony Movshon, who collaborates with Simoncelli. "But to make this fit to the biology is a unique skill."

Simoncelli even hopes that his work will lead to insights into consciousness. His peers say that's not arrogance but quiet confidence. "Eero's work is … both powerful and simple," says Matteo Carandini, a neuroscientist at the Smith-Kettlewell Eye Research Institute in San Francisco, California. "His group is the best thing around." Bruno Olshausen, a computational neuroscientist at the University of California (UC), Berkeley, adds that Simoncelli's work "has been very inspirational to lots of people, including me."

Brain as machine

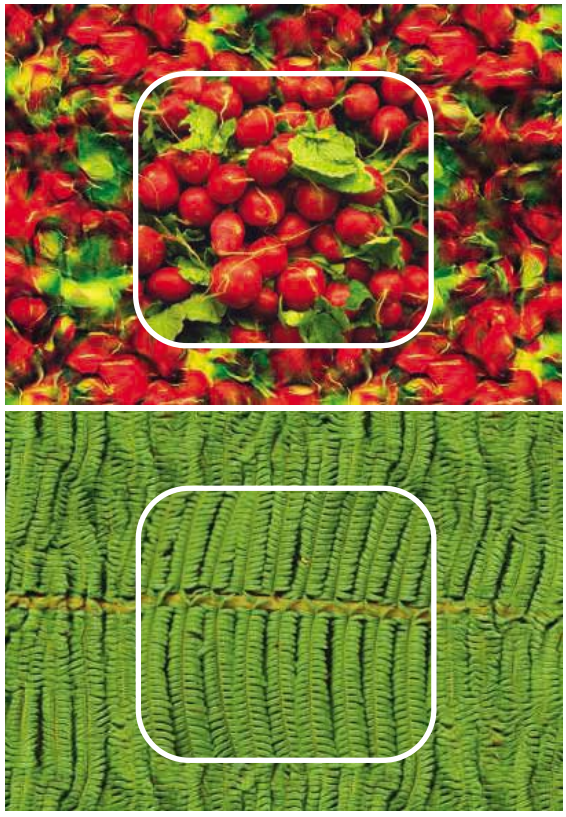
Simoncelli has wanted to study the brain since childhood. But he could not relate to—or remember—the piles of facts he was asked to learn in his introductory biology course at Harvard University. So he decided to major in physics instead of biology and later got a Ph.D. in electrical engineering while working in Edward "Ted" Adelson's visual science laboratory at the Massachusetts Institute of Technology. In his Ph.D. thesis, Simoncelli mathematically described a network of neurons that processes visual motion. His simulated brain cells performed computations mimicking the responses that neurophysiologists had recorded from cells in their laboratories. "He has brilliant intuitions about images and vision," Adelson says. "Combining engineering principles and biological insights, he's developed models of visual processing that are among the best in the world."

Simoncelli's Ph.D. analysis of visual motion captured a vexing oddity that other researchers had glossed over: the nonlinearity of vision-processing neurons. Engineers favor linear systems because they behave according to a simple law: If two stimuli are combined, the system's response to the combination is equal to the sum of its responses to each separate stimulus. By contrast, nonlinear systems generate more complex responses. "One of the reasons we have so much trouble trying to understand the brain is that it doesn't behave according to the rules of our standard engineering toolbox," Simoncelli says.

In the mid-1990s, as a computer science professor at the University of Pennsylvania, Simoncelli again embraced nonlinearity, producing a novel solution to a classic image-analysis problem: He identified a new set of mathematical regularities in the relations between the pixels that make up photographic scenes. His pixel analysis led to a state-of-the-art technique for compressing images and a method for eradicating visual noise that remains the best in the world as judged by experimental tests. Such a noise-removal technique might eventually be used to make crisper, filmlike image sensors in digital cameras or clear up pictures received from TV satellite dishes.

Bridging the gap

Next, Simoncelli wanted to link his image analysis to the human visual system. He hypothesized that evolution may have forced the brain to encode the visual world in the most efficient, mathematically optimal way. Using that concept, Simoncelli and his colleagues reported in 2001 that the nonlinear responses of neurons, such as those in the primary visual cortex at the back of the brain, are



Perception problem. By understanding mathematically how the brain perceives texture, Eero Simoncelli has developed software that can synthesize the textures in an image. It works best when the object has a regular pattern.

CREDIT: J. PORTILLA AND E.P. SIMONCELLI, *INT'L JOURNAL OF COMPUTER VISION* **40** 49–71 (2000)

well-matched to the statistical properties of the visual environment on Earth, that is, the mathematical patterns of lightness and darkness that recur in visual scenes. The result may help explain how evolution nudged certain visual neurons to be acutely sensitive to object edges and contours, for example.

Last year, Simoncelli and his colleagues reported building an image-compression tool based on his nonlinear model of cortical neurons. Simoncelli reasoned that if the brain's visual cortex is optimally efficient at processing images, it should also do a superior job of compressing them. What's more, any distortions introduced by his compression process should be tolerable. "If the cortical representation is like what's in our brains, we won't notice the difference," he says. Indeed, the new compression technique's performance far outstripped that of the JPEG standard.

Working with postdoc Javier Portilla, Simoncelli has similarly devised a novel mathematical description of how the brain achieves visual texture perception. That's led to a better way of synthesizing pictures—say, an image of a patch of a certain type of grass or cloth—that maintain a material's distinctive appearance. "The model provides a good description of what a person sees when looking at texture," Simoncelli says, adding that he and Portilla have tested it on an extensive number of texture images.

"It does something almost artistic," says UC Berkeley's Olshausen of Simoncelli's texture model. The model, Olshausen adds, not only points vision scientists to the essential properties of texture, but it also could be useful to filmmakers who would like to paint textures onto computer-generated images.

Despite the practical relevance of his work, Simoncelli has largely stayed within the ivory tower. Although he has filed for patents in the past, earning three, Simoncelli hasn't applied for any on his new texture work, or for his most recent noise-reduction and image-compression techniques. One reason, he says, is that patenting delays publication of his ideas. Moreover, applying for a patent on software, versus an actual device, "feels like playing the lottery because the chances are low that it's going to hold up. I don't care enough about money to make it a priority."

Visual insights. Simoncelli has explained why drivers speed in the fog and how the brain makes sense of moving objects.

CREDIT: NICOLAS BONNIER

In motion

Recently, Simoncelli has helped solve several riddles of motion perception. In the April issue of *Nature Neuroscience*, Simoncelli and his postdoc Alan Stocker explained the Thompson effect, in which motion seems to slow down when the visual landscape lacks contrast. This illusion, first described 25 years ago by psychologist Peter Thompson, helps account for why people drive too quickly in the fog. Simoncelli and Stocker asked five people to judge which of two computer-generated gratings looked like it was moving faster. The researchers varied the gratings' speed and contrast, and each volunteer was asked to make about 6000 separate judgments. Stocker and Simoncelli then analyzed the data using Bayesian statistics, a branch of mathematics that combines expectations with new information, and deduced each person's expectations from his or her speed perceptions. It turns out that people expect slow movement over fast, and that those expectations trump actual perceptions when the perceptual data are sketchy, as occurs in low-contrast situations (*Science*NOW, 21 March, sciencenow.sciencemag.org/cgi/content/full/2006/321/2)

Another 25-year-old motion mystery is also about to succumb to Simoncelli. Scientists have long known that cells in the primary visual cortex process pieces of a visual scene and that those pieces are then assembled into a greater whole by cells in other brain areas. But when an object is moving, it was not at all clear how a brain put the pieces together. Ever since his Ph.D. thesis, Simoncelli has worked on the calculations a computer should perform to mimic a system that can combine pieces of a moving image and spit out a coherent response. Again, he used Bayesian mathematics to try to make sense of people's perceptions of motion and the physiological data from visual neurons. He then mapped all of his computations onto a simulation of neuronal responses that starts in the retina and ends in the visual motion-processing region known as area MT.

In a paper to appear in *Nature Neuroscience* this fall, Simoncelli and Movshon along with postdocs Nicole Rust and Valerio Mante offer the first precise mathematical description of how cells in MT translate pieces of a moving scene into the movement of the whole. They vetted their model against new recordings from individual MT neurons in monkeys exposed to a specific set of stimuli: wiggling lines that look like the ripples on the surface of water. From the model, the researchers could extract biological information about MT cells, including which visual cortex cells feed into them. MT neurons are "profoundly nonlinear," Simoncelli says. "The model explains how that profound nonlinearity can arise from a cascade of very simple nonlinear steps."

Movshon, who did the experimental work buttressing the new model, describes Simoncelli's solution as "simple and elegant," and says the work also gives the field more sophisticated techniques for analyzing and extracting information from recordings of neuronal responses. Moreover, Simoncelli and his colleagues are putting the finishing touches on a set of algorithms that should help neuroscientists better interpret the flood of information that comes from recording large groups of neurons simultaneously in the retina, instead of one at a time as is traditionally done.

Ultimately, Simoncelli aims to put many of his individual findings, and those of his collaborators, into nothing less than a grand unified theory of visual motion perception. "In 10 years, I think we will have a clean computational model of motion," he predicts.

And if that wasn't ambitious enough, Simoncelli is digging for deeper truths. "As we build better descriptions of the brain and test them experimentally, we hope to arrive at fundamental principles that can explain all brain activity, from sensation to consciousness," he says. "That's going to help us understand who we are." Now that's a grand vision.

–INGRID WICKELGREN

REVIEW

Modeling Single-Neuron Dynamics and Computations: A Balance of Detail and Abstraction

Andreas V. M. Herz,[1]* Tim Gollisch,[2] Christian K. Machens,[3] Dieter Jaeger[4]

The fundamental building block of every nervous system is the single neuron. Understanding how these exquisitely structured elements operate is an integral part of the quest to solve the mysteries of the brain. Quantitative mathematical models have proved to be an indispensable tool in pursuing this goal. We review recent advances and examine how single-cell models on five levels of complexity, from black-box approaches to detailed compartmental simulations, address key questions about neural dynamics and signal processing.

A hundred years ago, Lapicque (*1*) proposed that action potentials are generated when the integrated sensory or synaptic inputs to a neuron reach a threshold value. This "integrate-and-fire" model remains one of the most influential concepts in neurobiology because it provides a simple mechanistic explanation for basic neural operations, such as the encoding of stimulus amplitude in spike frequency. However, advances in experimental technique have shown that the integrate-and-fire model is far from accurate in describing real neurons. Their morphology, composition of ionic conductances, and distribution of synaptic inputs generate a plethora of dynamical phenomena and support various fundamental computations (Table 1 and Table 2).

Understanding the dynamics and computations of single neurons and their role within larger neural networks is therefore at the core of neuroscience: How do single-cell properties contribute to information processing and, ultimately, behavior? Quantitative models address these questions, summarize and organize the rapidly growing amount and sophistication of experimental data, and make testable predictions. As single-cell models and experiments become more closely interwoven, the development of data analysis tools for efficient parameter estimation and assessment of model performance constitutes a central element of computational studies.

All these tasks require a delicate balance between incorporating sufficient details to account for complex single-cell dynamics and reducing this complexity to the essential characteristics to make a model tractable. The appropriate level of description depends on the particular goal of the model. Indeed, finding the best abstraction level is often the key to success. We highlight these aspects for five main levels (Fig. 1) of single-cell modeling.

Level I: Detailed Compartmental Models

Morphologically realistic models are based on anatomical reconstructions and focus on how the spatial structure of a neuron contributes to its dynamics and function. These models extend the cable theory of Rall, who showed mathematically that dendritic voltage attenuation spreads asymmetrically (*2*). This phenomenon allows dendrites to compute the direction of synaptic activation patterns, and thus provides a mechanism for motion detection (*3*). When voltage-dependent conductances are taken into account, numerical integration over the spatially discretized dendrite—the "compartmental model" (*3*)—is needed to solve the resulting high-dimensional system of equations.

For complex dendritic trees, more than 1000 compartments are required to capture the cell's specific electrotonic structure (e.g., to simulate spike backpropagation in pyramidal neurons) (*4*). Such detailed models also generate testable mechanistic hypotheses. For instance, simulations of Purkinje cells predicted that a net inhibitory synaptic current underlies specific spike patterns in vivo (*5*), in accordance with later experimental findings (*6*). In turn, even established models such as the thalamocortical neuron (*7*) are constantly improved by adding new biophysical details such as dendritic calcium currents responsible for fast oscillations (*8*).

A large body of morphologically realistic models demonstrates how spatial aspects of synaptic integration in dendrites support specific computations (Table 1 and Table 2), as discussed in various reviews (*9*, *10*). In pyramidal cells, for example, distal inputs are amplified via dendritic spikes or plateau potentials, supporting local coincidence detection and gain modulation. Dendritic inward currents play a major role in the control of spiking (*6*) or the modulation of responses to synchronous inputs (*11*). Such interactions among synaptic inputs, voltage-gated

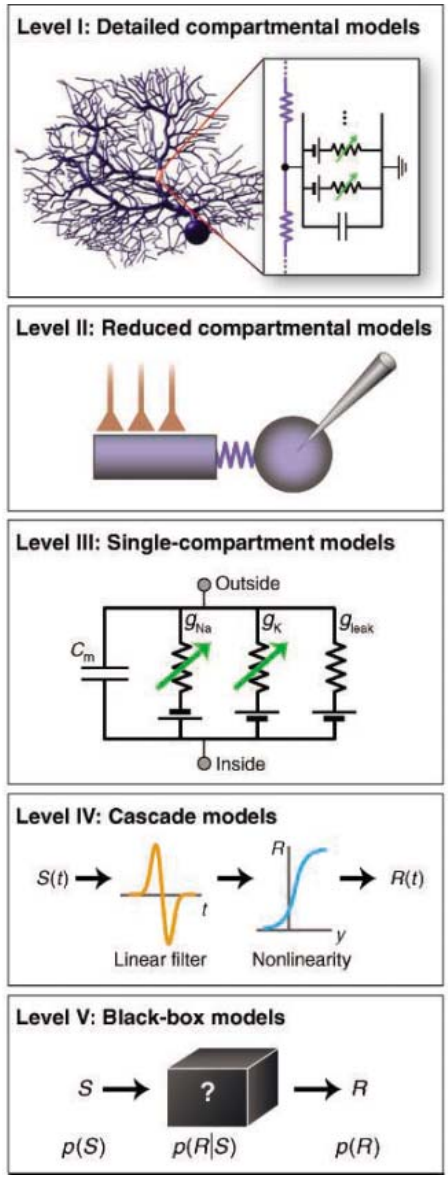


Fig. 1. Examples for five levels of single-cell modeling. **Level I**: Detailed compartmental model of a Purkinje cell. The dendritic tree is segmented into electrically coupled Hodgkin-Huxley–type compartments (level III). **Level II**: Two-compartment model as in (*23*). The dendrite receives synaptic inputs and is coupled to the soma where the neuron's response is generated. **Level III**: Hodgkin-Huxley model, the prototype of single-compartment models. The cell's inside and outside are separated by a capacitance C_m and ionic conductances in series with batteries describing ionic reversal potentials. Sodium and potassium conductances (g_{Na}, g_K) depend on voltage; the leak g_{leak} is fixed. **Level IV**: Linear-nonlinear cascade. Stimuli $S(t)$ are convolved with a filter and then fed through a nonlinearity to generate responses $R(t)$, typically time-dependent firing rates. **Level V**: Black-box model. Neglecting biophysical mechanisms, conditional probabilities $p(R|S)$ describe responses R for given stimuli S.

[1]Bernstein Center for Computational Neuroscience Berlin and Humboldt-Universität zu Berlin, Berlin 10099, Germany. [2]Department of Molecular and Cellular Biology, Harvard University, Cambridge, MA 02138, USA. [3]Cold Spring Harbor Laboratory, Cold Spring Harbor, NY 11724, USA. [4]Department of Biology, Emory University, Atlanta, GA 30322, USA.

*To whom correspondence should be addressed. E-mail: a.herz@biologie.hu-berlin.de

conductances, and spiking output can be specifically affected by dendritic branching structures (*12*); axonal geometries, on the other hand, influence activity-dependent branch point failures and may thus implement filter and routing operations on the neuron's output side (*13*).

Finally, detailed spatial representations help predict the effects of extracellular electrical stimulations. This is of great interest for deep-brain stimulation used in the treatment of Parkinson's disease (*14*) and underscores the need for morphologically realistic models.

Table 1. Information processing in single neurons: Basic computations that follow from generic neuronal properties.

Computation	Biophysical mechanism	Model level
Addition or subtraction	Dendritic summation of excitatory and/or inhibitory inputs (*3*)	I, II
Subtraction	Shunting inhibition plus integrate-and-fire mechanism (*76*)	I, II
Multiplication or division	Synaptic interaction (*67*, *77*)	I, II
	Gain modulation via synaptic background noise (*35*, *36*)	I, II
High-pass filter	Firing rate adaptation (*32*)	III
Low-pass filter	Passive membrane properties (*28*)	I, II, III
Toggle switch	Bistable spike generation (*30*)	III

Level II: Reduced Compartmental Models

Although detailed compartmental models can approximate the dynamics of single neurons quite well, they suffer from several drawbacks. Their high dimensionality and intricate structure rule out any mathematical understanding of their emergent properties. Detailed models are also computationally expensive and are thus not well suited for large-scale network simulations. Reduced models with only one or few dendritic compartments overcome these problems and are often sufficient to understand somatodendritic interactions that govern spiking (*15*) or bursting (*16*).

A well-matched task for such models is to relate behaviorally relevant computations on

Table 2. Information processing in single neurons: Task-specific computations of direct behavioral relevance.

Biological goal	Computation	Biophysical mechanism	Model level	Experimental systems
Collision avoidance	Multiplication: object size x times angular velocity y	$xy = \exp(\log x + \log y)$ via input nonlinearity (log), dendritic summation (+), and output nonlinearity (exp)	III	Lobula giant movement detector in locusts (*52*)
Sound localization	Logical AND: comparison of interaural time difference	Coincidence detection of two spikes, lagged by different axon delays (*17*, *18*)	II	Binaural neurons in the auditory brainstem (*17*)
Motion detection	Logical AND or AND-NOT: comparison of spatially adjacent but temporally shifted local light intensities	Coincidence detection of one lagged (axonal delay) and one nonlagged spike (*59*)	IV	Peripheral neurons in the fly visual system [see, e.g., (*59*, *60*)]
		Nonlinear dendritic processing (*3*, *78*)	I, II	Retinal amacrine and ganglion cells (*79*, *80*)
Motion anticipation	Linear filtering with negative feedback	Adaptation of neuronal gain	IV	Salamander and rabbit retinal ganglion cells (*81*)
Intensity-invariant recognition of analog patterns	Separation of pattern identity and pattern intensity; subsequent comparison with stored template	Transformation: local stimulus intensity mapped to spike time using subthreshold membrane potential oscillations; readout: coincidence detection (*82*)	III, IV	Insect and vertebrate olfactory neurons, in particular in antennal lobe and olfactory bulb, respectively (*83*)
Short-term memory	Temporal integration or storage	Dendritic Ca waves (*20*)	II	Layer V neurons in entorhinal cortex (*19*)
		Transitions between two Ca-conductance states (*21*)	II	Layer V neurons in entorhinal cortex (*21*)
Time interval prediction	Temporal integration or storage	Calcium dynamics with positive feedback (*84*)	III	Climbing activity in prefrontal neurons (*85*)
Redundancy reduction	Subtraction: local signal minus background signal	Dendritic summation (*3*)	IV	Center-surround receptive fields in the visual system (*57*)
Efficient coding in variable environment	Modification of tuning curve to track time-varying stimulus ensemble	Adaptation of single-cell input-output function (*23*, *58*)	II, IV, V	Motion-sensitive H1 neuron in the fly visual system (*58*, *60*)
		Consequence (*60*) of Reichardt motion detector circuit (*59*)	IV, V	

various time scales to salient features of neural structure and dynamics. For example, the detection of binaural time differences within Jeffress' time-delay framework (*17*) has been explained in a three-compartment model of bipolar cells by local nonlinear input interactions and the fact that each of the two dendrites provides a sink for inputs received by the other dendrite (*18*). Computations involving short-term memory may rely in part on the existence of multiple stable firing rates in single neurons. Reduced compartmental models suggest that calcium currents are essential for this phenomenon (*19*), through dendritic calcium wavefronts (*20*) or transitions between different conductance states (*21*). On longer time scales, neurons self-adjust their activity patterns, both during development and after external perturbations (*22*). Simulations with a two-compartment model show that such homeostatic plasticity can follow from cellular "learning" rules that recalibrate dendritic channel densities to yield optimal spike encoding of synaptic inputs (*23*).

For large-scale network studies, reduced compartmental models offer a good compromise between realism and computational efficiency. For example, a simulation involving several classes of multicompartmental cortical and thalamic neurons and a total of more than 3000 cells demonstrates that gap junctions are instrumental for cortical gamma oscillations (*24*). A slightly less complex network with two-compartment neurons reproduces slow-wave sleep oscillations (*25*). Clearly, the challenge for all such studies is to find the least complex neuron models with which the observed phenomena can still be recreated (*26*).

Level III: Single-Compartment Models

Single-compartment models such as the classic Hodgkin-Huxley model (*27*) neglect the neuron's spatial structure and focus entirely on how its various ionic currents contribute to subthreshold behavior and spike generation. These models have led to a quantitative understanding of many dynamical phenomena including phasic spiking, bursting, and spike-frequency adaptation (*28*).

Systematic mathematical reductions of Hodgkin-Huxley–type models and subsequent bifurcation and phase-plane analysis (*29*, *30*) explain why, for example, some neurons resemble integrate-and-fire elements or why the membrane potential of others oscillates in response to current injections enabling a "resonate-and-fire" behavior. They also show which combination of dynamical variables governs the threshold operation (*31*) and how adaptation (*32*) and spike-generation mechanisms (*33*) influence spike trains (Fig. 2).

Spike generation is not a deterministic process. The stochastic dynamics of ion channels generate voltage noise that limits the reliability and precision of spikes (*34*). Background synaptic noise (*35*), on the other hand, can modulate the neural gain without changing spike variability or mean firing rates (*36*). But even without intrinsic noise, the all-or-none characteristics of spike generation amplify the input variability (*37*)—perhaps this is the price of long-distance communication.

More than 50 years after Hodgkin and Huxley analyzed the squid axon, simple neuron models still offer surprises, as these findings show. A recent study even indicates that the standard Hodgkin-Huxley formalism does not explain the sharp kink at the onset of cortical spikes (*38*). Its mechanistic origin and functional consequences require further investigation.

Level IV: Cascade Models

Whereas models incorporating specific ionic currents or morphological details are needed to investigate the biophysics of single neurons, modeling on a more conceptual level allows one to directly address their computations. To this end, cascade models based on a concatenation of mathematical primitives, such as linear filters, nonlinear transformations, and random processes, present an excellent framework for distilling key processing steps from measured data.

Consider, for example, a model that first convolves its time-varying input with a linear filter and then applies a rectifying nonlinearity. In studies of sensory systems, this simple structure is often considered as the canonical model for the receptive field of a neuron and the transformation of its internal activation state into a firing rate. The appeal of this linear-nonlinear (LN) cascade stems from its conceptual simplicity and the fact that, for white-noise stimulation, it can be easily fitted to experimental data by correlating response and stimulus (*39*). Recent studies even demonstrate that LN cascades can be obtained under far more naturalistic stimulation (*40*, *41*).

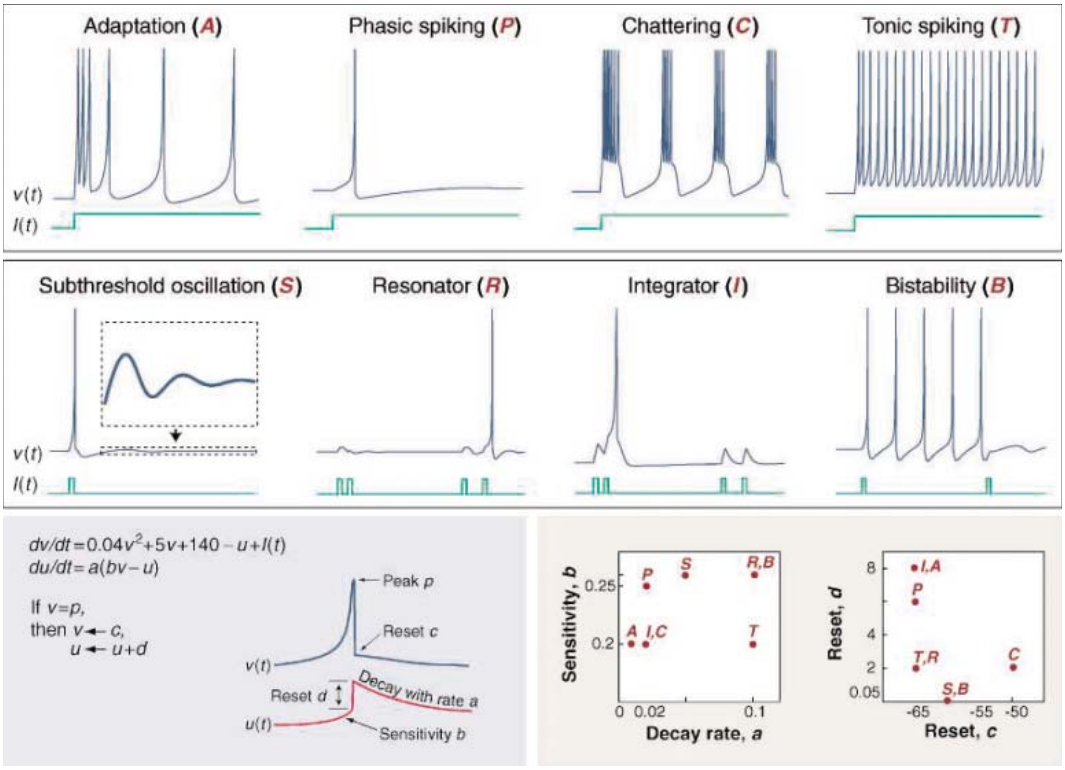


Fig. 2. Diversity of neural response patterns. As illustrated in the top row, neurons can respond with rather different spike-train patterns to identical step currents. For time-varying inputs (middle row), the computational power of even simple single-neuron models becomes apparent: A first current pulse might trigger a subthreshold oscillation. Only if a second pulse arrives at the right phase of this oscillation is a spike triggered through resonance. An integrator, on the other hand, is driven most effectively by quickly succeeding pulses. Finally, a bistable cell can realize a toggle-switch. These phenomena [and many more; see (*30*)] are exhibited by the same point-neuron model: Its time evolution (bottom row, left) is derived from Hodgkin-Huxley–type dynamics; involves the membrane potential v and a slower auxiliary variable u; and generates the different responses for specific values (right) of the parameters c (reset of voltage v with peak p) and a, b, and d (decay rate, sensitivity, and reset of the auxiliary variable u). Figure courtesy of E. Izhikevich.

Cascade models have a long tradition in the investigation of the visual system. More recently, they have been used to assess neuronal sensitivity for different stimulus features and have helped to elucidate the simultaneous adaptation to mean light intensity and light contrast (*42*) and the generic nature of adaptation in the retina (*43*). New analysis tools have opened up the possibility of using multiple parallel linear filters in an LN cascade to investigate, for example, complex cells in visual cortex (*44*) and thus improve on classical energy-integration models (*45*).

Extending LN cascades allows one to capture additional neural characteristics while retaining the ability to fit these more complex models to experimental data. To reveal filter mechanisms that are otherwise hidden by spike-time jitter, one may append a noise process to the cascade (*46*, *47*) or measure spike probabilities instead of spike times (*48*). For the latter method, temporal resolution is limited only by the precision of stimulus presentation so that parameters of more elaborate models (e.g., LNLN cascades) can be obtained.

The analog output of traditional cascade models describes a firing rate. An important conceptual extension is therefore achieved by adding an explicit spike generation stage. Using a fixed firing threshold and feedback mimicking neural refractoriness (*49*), this has led to a successful model of spike timing in early vision (*50*). Even when augmented with an integrate-and-fire mechanism and intrinsic bursting, this model structure still allows generic fits to measured spike trains (*51*).

Cascade models can also directly translate into specific computations: Experiments indicate that in locusts, an identified neuron multiplies the visual size x and angular velocity y of an object while tracking its approach (*52*). The nearly exponential shape of this neuron's output curve suggests that logarithmic transforms of x and y are summed on the dendrite and then passed through the output nonlinearity, implementing the multiplicative operation as an NLN cascade via the identity $\exp(\log x + \log y) = xy$.

Despite their success, simple model structures have their limitations—especially when applied to neurons far downstream from the sensory periphery and when aimed at generalizing over different stimulus types—because additional nonlinear dynamics, negligible within a specific stimulation context, affect the transition between different experimental conditions (*53*, *54*). In specific cases, however, LN models yield accurate information-theoretical descriptions of neuronal responses (*55*).

Level V: Black-Box Models

Last but not least, one may want to understand and quantify the signal-processing capabilities of a single neuron without considering its biophysical machinery. This approach may reveal general principles that explain, for example, where neurons place their operating points and how they alter their responses when the input statistics are modified.

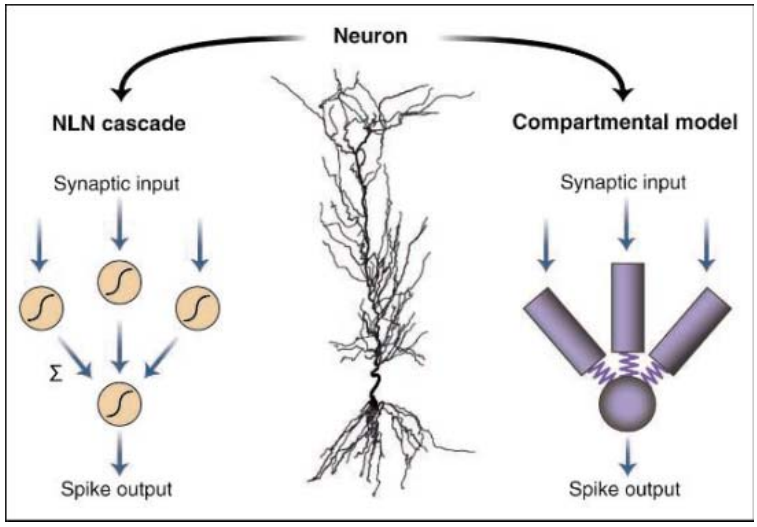


Fig. 3. Single-neuron computation. The neuron in the center (*86*) can be approximated by an NLN cascade (left) for stationary inputs (*67*), or, more generally, by a compartmental model (right). The cascade (level IV) is equivalent to a two-layer feedforward network and shows that under a firing-rate assumption, a single neuron may perform the function of an entire artificial neural net. Electrical couplings within compartmental models (levels I and II) are bidirectional. The right model therefore corresponds to a feedback network and can exhibit persistent activity, hysteresis, periodic oscillations, and even chaos. These phenomena are impossible in feedforward systems and may support complex computations in the time domain. The relevance of either model depends on the statistics of synaptic inputs (i.e., on the neural code of the investigated brain area).

For such questions about neural efficiency and adaptability, a neuron is best regarded as a black box that receives a set of time-dependent inputs—sensory stimuli or spike trains from other neurons—and responds with an output spike train. To account for cell-intrinsic noise, it is necessary to characterize the input-output relation by a probability distribution, $p(R|S)$, which measures the probability that response R occurs when stimulus S is presented.

Although models on levels I to IV make specific assumptions about neural processes and hence about the functional form of $p(R|S)$, such assumptions can be overly restrictive at level V. Here, it is often advantageous to work with nonparametric estimates of $p(R|S)$ that are directly taken from the measured data. Such models have, for example, been used to estimate the information that the spike train of a neuron conveys about its inputs and have revealed that sensory neurons operate highly efficiently, often close to their physical limits (*56*). Indeed, Barlow's "efficient coding hypothesis" suggests that neurons optimize the information about frequently occurring stimuli (*57*).

Theoretical studies have shown how individual neurons may shift their input-output curves to reach that goal (*23*). Moreover, recordings of a motion-sensitive neuron in the fly visual system reveal that adaptation can modify a neuron's input-output function to maximize information about time-varying sensory stimuli (*58*). In this case, however, it is possible that the adaptive mechanism is not implemented on the single-cell level but instead results from the underlying multicellular Reichardt motion detection circuitry (*59*, *60*). Similar ambiguities between single-cell and network adaptation exist in the auditory midbrain (*61*).

Evolutionary adaptations may not be guided to optimize the information about all natural stimuli. In acoustic communication systems, for example, neural responses are well matched to particular behaviorally relevant subensembles. Most likely, stimuli from those ensembles were selected as communication signals because they lead to efficient neural representations (*62*, *63*).

Challenges

"A good theoretical model of a complex system should be like a good caricature: it should emphasize those features which are most important and should downplay the inessential details. Now the only snag with this advice is that one does not really know which are the inessential details until one has understood the phenomena under study" (*64*).

This general dilemma, formulated by the physicist Frenkel almost a century ago, applies in particular to the single neuron. Which details of ionic conductances and morphology are relevant for particular aspects of its cell type–specific or individual dynamics? How do these dynamics contribute to the neuron's information processing? Identification of a fundamental computation performed by the neuron (Table 1 and Table 2) may help address these questions. Brain function, however, relies on the interplay of hundreds to billions of neurons that are arranged in specialized modules on multiple anatomical hierarchies. Even

today, it remains unclear which level of single-cell modeling is appropriate to understand the dynamics and computations carried out by such large systems. However, only by understanding how single cells operate as part of a network (*35*) can we assess their coding and thus the level of detail required for modeling. For example, most network models use point-neuron models (*65*), whereas several aspects of brain function require multicompartmental models (*24*, *25*).

It has thus become increasingly clear that a thorough understanding of single-neuron function can be obtained only by relating different levels of abstraction. Trying to incorporate every biological detail of the investigated neuron is likely to obscure the focus on the essential dynamics, whereas limiting investigations to highly abstract processing schemes casts doubt on the biological relevance of specific findings. Help may come from analyzing the transition between different modeling levels. Interesting connections have been drawn, for example, by transforming a Hodgkin-Huxley–type model (level III) into a phenomenological firing rate description (*66*) or a cascade on level IV (*31*). And the integrative properties of dendritic trees as evolved as those of pyramidal cells can be captured by a two-layer feedforward network (i.e., an NLN cascade) (level IV), at least for stationary stimuli (*67*). For nonstationary stimuli, however, the cascade fails (Fig. 3). This underscores the need to alternate between different levels of single-neuron models in close connection with considerations about the neural codes of larger cell populations.

Deriving model parameters from experimental data brings about its own collection of problems: How should we deal with the cell-to-cell variability of parameter values? The common resort, population averaging, can be misleading because the dynamical behavior of single-cell models is, in general, not a monotone function of their parameters; the mean behavior within a class of models may strongly differ from that of a model with mean parameter values (*68*), and nearly identical dynamical characteristics may be implemented by rather different parameter combinations (*69*). With increasing model complexity, the number of parameters to be estimated increases to such an extent that they must be taken from different cells or even different preparations, further lowering the model's trustworthiness. Furthermore, models are often calibrated using in vitro data, yet they are designed to capture the neural dynamics and computations of behaving animals.

Conclusions

There is no general solution for any of these challenges. Iterating the loop of model prediction, experimental test, and model adjustment is an obvious strategy for stepwise progress. One should be aware, however, that elaborate single-cell models are not sufficiently constrained by data, nor is there any guarantee that crucial components of the real biological neuron are already included. Wrong models may therefore be falsely "verified," and long-term progress may require many iterations of the model-experiment loop until an incorrect assumption is eventually falsified.

There is good news, too: The rapid development of experimental tools to study single neurons in vivo (*70*) will generate data urgently needed to advance quantitative models. With powerful computers tightly integrated in modern laboratories, advanced on-line techniques such as the "dynamic clamp" (*71*) will be used routinely in the future. In this technique, voltage-gated conductances that cannot be selectively blocked by pharmacological agents are counterbalanced by currents that are artificially generated using the neuron's present state. This approach has clarified, for example, the influence of persistent sodium currents on spike generation (*72*). To mimic in vivo input patterns during in vitro experiments, synaptic conductances can be inserted with a dynamic clamp (*6*, *36*, *73*). Adaptive stimulations with real-time data analysis can also be used to optimize recording times, allowing one to extend traditional concepts such as "best stimulus" to the information-theoretic level (*62*).

These developments show that the divide between experiment and theory is disappearing. There is also a change in attitude reflected by various international initiatives (*74*, *75*): More and more experimentalists are willing to share their raw data with modelers. Many modelers, in turn, make their computer codes available. Both movements will play a key role in solving the many open questions of neural dynamics and information processing—from single cells to the entire brain.

References and Notes

1. L. Lapicque, *J. Physiol. Pathol. Gen.* **9**, 620 (1907).
2. W. Rall, *Exp. Neurol.* **1**, 491 (1959).
3. W. Rall, in *Neural Theory and Modeling*, R. F. Reiss, Ed. (Stanford Univ. Press, Stanford, CA, 1964), pp. 73–97.
4. N. L. Golding, W. L. Kath, N. Spruston, *J. Neurophysiol.* **86**, 2998 (2001).
5. D. Jaeger, E. De Schutter, J. M. Bower, *J. Neurosci.* **17**, 91 (1997).
6. D. Jaeger, J. M. Bower, *J. Neurosci.* **19**, 6090 (1999).
7. A. Destexhe, M. Neubig, D. Ulrich, J. Huguenard, *J. Neurosci.* **18**, 3574 (1998).
8. P. A. Rhodes, R. Llinas, *J. Physiol.* **565**, 765 (2005).
9. C. Koch, I. Segev, *Nat. Neurosci.* **3**, 1171 (2000).
10. M. London, M. Häusser, *Annu. Rev. Neurosci.* **28**, 503 (2005).
11. F. Santamaria, J. M. Bower, *J. Neurophysiol.* **93**, 237 (2005).
12. A. T. Schaefer, M. E. Larkum, B. Sakmann, A. Roth, *J. Neurophysiol.* **89**, 3143 (2003).
13. D. Debanne, *Nat. Rev. Neurosci.* **5**, 304 (2004).
14. C. C. McIntyre, W. M. Grill, D. L. Sherman, N. V. Thakor, *J. Neurophysiol.* **91**, 1457 (2004).
15. A. S. Kuznetsov, N. J. Kopell, C. J. Wilson, *J. Neurophysiol.* **95**, 932 (2006).
16. A. Kepecs, X. J. Wang, J. Lisman, *J. Neurosci.* **22**, 9053 (2002).
17. L. A. Jeffress, *J. Comp. Physiol. Psychol.* **41**, 35 (1948).
18. H. Agmon-Snir, C. E. Carr, J. Rinzel, *Nature* **393**, 268 (1998).
19. A. V. Egorov, B. N. Hamam, E. Fransen, M. E. Hasselmo, A. A. Alonso, *Nature* **420**, 173 (2002).
20. Y. Loewenstein, H. Sompolinsky, *Nat. Neurosci.* **6**, 961 (2003).
21. E. Fransen, B. Tahvildari, A. V. Egorov, M. E. Hasselmo, A. A. Alonso, *Neuron* **49**, 735 (2006).
22. G. G. Turrigiano, S. B. Nelson, *Nat. Rev. Neurosci.* **5**, 97 (2004).
23. M. Stemmler, C. Koch, *Nat. Neurosci.* **2**, 521 (1999).
24. R. D. Traub *et al.*, *J. Neurophysiol.* **93**, 2194 (2005).
25. M. Bazhenov, I. Timofeev, M. Steriade, T. J. Sejnowski, *J. Neurosci.* **22**, 8691 (2002).
26. P. F. Pinsky, J. Rinzel, *J. Comp. Neurosci.* **1**, 39 (1994).
27. A. L. Hodgkin, A. F. Huxley, *J. Physiol.* **117**, 500 (1952).
28. C. Koch, *Biophysics of Computation: Information Processing in Single Neurons* (Oxford Univ. Press, New York, 1998).
29. J. Rinzel, G. B. Ermentrout, in *Methods in Neuronal Modeling: From Synapses to Networks*, C. Koch, I. Segev, Eds. (MIT Press, Cambridge, MA, 1989), pp. 135–169.
30. E. M. Izhikevich, *IEEE Trans. Neur. Networks* **15**, 1063 (2004).
31. B. Aguera y Arcas, A. L. Fairhall, W. Bialek, *Neural Comput.* **15**, 1715 (2003).
32. J. Benda, A. V. M. Herz, *Neural Comput.* **15**, 2523 (2003).
33. N. Fourcaud-Trocmé, D. Hansel, C. van Vreeswijk, N. Brunel, *J. Neurosci.* **23**, 11628 (2003).
34. E. Schneidman, B. Freedman, I. Segev, *Neural Comput.* **10**, 1679 (1998).
35. A. Destexhe, D. Contreras, *Science* **314**, 85 (2006).
36. F. S. Chance, L. F. Abbott, A. D. Reyes, *Neuron* **35**, 773 (2002).
37. M. Carandini, *PLoS Biol.* **2**, e264 (2004).
38. B. Naundorf, F. Wolf, M. Volgushev, *Nature* **440**, 1060 (2006).
39. E. J. Chichilnisky, *Network Comput. Neural Syst.* **12**, 199 (2001).
40. L. Paninski, *Neural Comput.* **15**, 1191 (2003).
41. T. O. Sharpee *et al.*, *Nature* **439**, 936 (2006).
42. V. Mante, R. A. Frazor, V. Bonin, W. S. Geisler, M. Carandini, *Nat. Neurosci.* **8**, 1690 (2005).
43. T. Hosoya, S. A. Baccus, M. Meister, *Nature* **436**, 71 (2005).
44. N. C. Rust, O. Schwartz, J. A. Movshon, E. P. Simoncelli, *Neuron* **46**, 945 (2005).
45. E. H. Adelson, J. R. Bergen, *J. Opt. Soc. Am. A* **2**, 284 (1985).
46. Z. N. Aldworth, J. P. Miller, T. S. Gedeon, G. I. Cummins, A. G. Dimitrov, *J. Neurosci.* **25**, 5323 (2005).
47. T. Gollisch, *Network Comput. Neural Syst.* **17**, 103 (2006).
48. T. Gollisch, A. V. M. Herz, *PLoS Biol.* **3**, e8 (2005).
49. W. Gerstner, J. L. van Hemmen, *Network Comput. Neural Syst.* **3**, 139 (1992).
50. J. Keat, P. Reinagel, R. C. Reid, M. Meister, *Neuron* **30**, 803 (2001).
51. J. W. Pillow, L. Paninski, V. J. Uzzell, E. P. Simoncelli, E. J. Chichilnisky, *J. Neurosci.* **25**, 11003 (2005).
52. F. Gabbiani, H. G. Krapp, C. Koch, G. Laurent, *Nature* **420**, 320 (2002).
53. C. K. Machens, M. S. Wehr, A. M. Zador, *J. Neurosci.* **24**, 1089 (2004).
54. M. Carandini *et al.*, *J. Neurosci.* **25**, 10577 (2005).
55. T. L. Adelman, W. Bialek, R. M. Olberg, *Neuron* **40**, 823 (2003).
56. A. Borst, F. E. Theunissen, *Nat. Neurosci.* **2**, 947 (1999).
57. H. B. Barlow, in *Sensory Communication*, W. A. Rosenblatt, Ed. (MIT Press, Cambridge, MA, 1961), pp. 217–234.
58. A. L. Fairhall, G. D. Lewen, W. Bialek, R. R. D. van Steveninck, *Nature* **412**, 787 (2001).
59. B. Hassenstein, W. Z. Reichardt, *Z. Naturforsch.* **11b**, 513 (1956).
60. A. Borst, V. L. Flanagin, H. Sompolinsky, *Proc. Natl. Acad. Sci. U.S.A.* **102**, 6172 (2005).
61. I. Dean, N. S. Harper, D. McAlpine, *Nat. Neurosci.* **8**, 1684 (2005).
62. C. K. Machens, T. Gollisch, O. Kolesnikova, A. V. M. Herz, *Neuron* **47**, 447 (2005).
63. E. C. Smith, M. S. Lewicki, *Nature* **439**, 978 (2006).
64. M. E. Fisher, in *Springer Lecture Notes in Physics, Vol. 186*, F. J. Hahne, Ed. (Springer, Berlin, 1983), pp. 1–139.
65. T. P. Vogels, K. Rajan, L. F. Abbott, *Annu. Rev. Neurosci.* **28**, 357 (2005).
66. O. Shriki, D. Hansel, H. Sompolinsky, *Neural Comput.* **15**, 1809 (2003).

67. P. Poirazi, T. Brannon, B. W. Mel, *Neuron* **37**, 989 (2003).
68. J. Golowasch, M. S. Goldman, L. F. Abbott, E. Marder, *J. Neurophysiol.* **87**, 1129 (2002).
69. M. S. Goldman, J. Golowash, E. Marder, L. F. Abbott, *J. Neurosci.* **21**, 5229 (2001).
70. M. Brecht *et al.*, *J. Neurosci.* **24**, 9223 (2004).
71. A. A. Prinz, L. F. Abbott, E. Marder, *Trends Neurosci.* **27**, 218 (2004).
72. K. Vervaeke, H. Hu, L. J. Graham, J. F. Storm, *Neuron* **49**, 257 (2006).
73. J. Wolfart, D. Debay, G. LeMasson, A. Destexhe, T. Bal, *Nat. Neurosci.* **8**, 1760 (2005).
74. Neuroscience Database Gateway (http://ndg.sfn.org).
75. International Neuroinformatics Coordinating Facility (www.incf.org).
76. G. R. Holt, C. Koch, *Neural Comput.* **9**, 1001 (1997).
77. B. W. Mel, *Neural Comput.* **4**, 502 (1992).
78. C. Koch, T. Poggio, V. Torre, *Proc. Natl. Acad. Sci. U.S.A.* **80**, 2799 (1983).
79. L. J. Borg-Graham, *Nat. Neurosci.* **4**, 176 (2001).
80. T. Euler, P. B. Detwiler, W. Denk, *Nature* **418**, 845 (2002).
81. M. J. Berry, I. H. Brivanlou, T. A. Jordan, M. Meister, *Nature* **398**, 334 (1999).
82. J. J. Hopfield, *Nature* **376**, 33 (1995).
83. T. W. Margrie, A. T. Schaefer, *J. Physiol.* **546**, 363 (2003).
84. D. Durstewitz, *J. Neurosci.* **23**, 5342 (2003).
85. G. Rainer, S. C. Rao, E. K. Miller, *J. Neurosci.* **19**, 5493 (1999).
86. R. C. Cannon, D. A. Turner, G. K. Pyapali, H. V. Wheal, *J. Neurosci. Methods* **84**, 49 (1998).
87. We thank M. Brecht, A. Destexhe, F. Gabbiani, E. Izhikevich, and A. Roth for helpful comments on earlier versions of this review, and E. Izhikevich for providing Fig. 2.

10.1126/science.1127240

REVIEW

Neuronal Computations with Stochastic Network States

Alain Destexhe[1]* and Diego Contreras[2]

Neuronal networks in vivo are characterized by considerable spontaneous activity, which is highly complex and intrinsically generated by a combination of single-cell electrophysiological properties and recurrent circuits. As seen, for example, during waking compared with being asleep or under anesthesia, neuronal responsiveness differs, concomitant with the pattern of spontaneous brain activity. This pattern, which defines the state of the network, has a dramatic influence on how local networks are engaged by inputs and, therefore, on how information is represented. We review here experimental and theoretical evidence of the decisive role played by stochastic network states in sensory responsiveness with emphasis on activated states such as waking. From single cells to networks, experiments and computational models have addressed the relation between neuronal responsiveness and the complex spatiotemporal patterns of network activity. The understanding of the relation between network state dynamics and information representation is a major challenge that will require developing, in conjunction, specific experimental paradigms and theoretical frameworks.

Brain operations are embedded in a continuous stream of complex spontaneous activity that interacts nonlinearly with incoming sensory inputs, outgoing motor commands, and internal association processes. Spontaneous brain activity refers to ongoing network activity not dominated by any particular single sensory input. Spontaneous brain activity is generated by the combination of intrinsic electrophysiological properties of single neurons (*1*) and synaptic interactions in networks (*2*); it is dependent on the level of activation of neuromodulatory systems (*3*, *4*) and is correlated with the functional state of the brain (*2*). Most of the existing knowledge about the relation between neuronal responsiveness and spontaneous brain activity comes from the comparison between waking and sleep states (*5*). However, even within the stable state of waking, subtle variations in the spatiotemporal pattern of network activation strongly influence information processing, and vice versa, sensory inputs modify ongoing activity. Such interplay between intrinsically generated activity and its modulation by external input is at the very core of the mechanisms by which the brain represents the external world and elaborates successful response strategies. The complexity of network dynamics is beyond the reach of current recording methods and requires appropriate computational methods carefully constrained by biological data. Predictions from current modeling efforts are a critical guide for designing new experimental approaches.

[1]Integrative and Computational Neuroscience Unit (UNIC), CNRS, Gif sur Yvette, France. [2]Department of Neuroscience, University of Pennsylvania School of Medicine, Philadelphia, PA 19104, USA.

*To whom correspondence should be addressed. E-mail: Destexhe@iaf.cnrs-gif.fr.

Experimental Characterization of Intrinsic Dynamics in Neocortex

Understanding the neuronal mechanisms of spontaneous brain activity is of critical importance in understanding its role in information processing. For example, the cellular mechanisms of synchronized oscillations during sleep and anesthesia explain why neural responsiveness is reduced during those states (*2*). However, much less is known about the complex intrinsic dynamics that characterize the spontaneous activity during the waking state. It is during the waking state that response variability and the spatiotemporal patterns of network activation are key elements of the brain operations that generate adaptive behavior.

The spontaneous activity recorded in the electroencephalogram (EEG) from cortex and thalamus varies greatly between waking and sleep states. During sleep, the EEG is dominated by large-amplitude waves with high temporal and spatial coherence (Fig. 1A), and most of its spectral power is below 15 Hz (*4*). Rhythmic components are prevalent, although they are highly aperiodic and interspersed with nonrhythmic large-amplitude waves. Intracellular recordings in vivo demonstrate large variations in membrane potential (V_m) occurring synchronously across large populations (*5*, *6*).

In contrast, upon awakening or during rapid eye movement (REM) sleep (also termed brain activated states), EEG spontaneous activity is characterized by low-amplitude waves, with low spatial and temporal coherence and high spatiotemporal complexity (Fig. 1B), not dominated by any identifiable pattern (*4*). The spectral power of the activated EEG is characterized by frequencies above 15 Hz. Intracellular recordings in vivo during activated states demonstrate absence of slow oscillations or any large V_m fluctuations characteristic of sleep (*7*). Instead, cortical and thalamic neurons show a stable resting V_m at a depolarized level close to firing threshold and a noisy, highly irregular pattern of background synaptic activity (*7*) (Fig. 1C). Interspersed within the synaptic background activity, there are short bouts of fast (20 to 80 Hz) oscillations, which last a few tens of milliseconds and which are occasionally crowned by spikes (*8*). Therefore, fast oscillations in cortical and thalamic networks are different from the intrinsically generated, well-organized, and stable subthreshold oscillations in highly rhythmic structures such as the inferior olive (*9*). Fast oscillations also appear in relation to sensory stimuli and have been proposed to subserve a coordinating function among neuronal groups

representing similar stimulus features [reviewed in (*10*)]. How the same oscillations that appear spontaneously as part of the background activity (*8*) are also steered to fulfill such function is unknown.

Relation Between Spontaneous Activity and Neuronal Responsiveness

The relation between spontaneous activity and neuronal and network responsiveness has been mainly studied by observing the dramatic changes that take place during the transition between the behavioral states of waking and sleep or during variations in the level of anesthesia (Fig. 1D). A plethora of functional studies in the visual (*11*–*16*), somatosensory (*17*), auditory (*18*–*20*), and olfactory (*21*) systems have shown that slow, high-amplitude activity in the EEG is associated with reduced neuronal responsiveness and neuronal selectivity. [For an extensive review of the literature and a historical perspective, see (*5*).]

Cellular studies in vivo and in vitro have shown how such changes in responsiveness come about. During the transition to sleep or anesthesia, cortical and thalamic cells progressively hyperpolarize, thus distancing the membrane from spike threshold and decreasing excitability. In addition, hyperpolarization brings the membrane potential to the activation range of intrinsic currents underlying burst firing, particularly in thalamic cells. Because of its all-or-none behavior and its long refractory period, thalamic bursting is incompatible with the relay function that characterizes activated states and thus act as the first gate of forebrain deafferentation, i.e., blockade of ascending sensory inputs (*22*, *23*). Synchronized inhibitory inputs during sleep oscillations further hyperpolarize cortical and thalamic neurons and generate large membrane shunting, resulting in a dramatic decrease in responsiveness and a large increase in response variability. Finally, highly synchronized patterns of rhythmic activity (*24*) dominate neuronal membrane behavior and render the network unreliable and less responsive to inputs. Taken together, the above mechanisms result in the functional brain deafferentation that characterizes sleep and anesthesia (*2*, *22*).

In contrast, during waking and REM sleep, a depolarized stable resting V_m close to spike threshold allows neurons to respond to inputs more reliably and with less response variability. However, the detailed understanding of the cellular mechanisms underlying the changes in processing state between waking and sleep or anesthesia is not enough to explain an important paradox posed by the two activated brain states. Despite their striking electrophysiological similarity at the intracellular and EEG levels (*7*) and the often enhanced evoked potentials during REM (*25*, *26*), waking and REM are diametrically opposite behavioral states (*27*), because REM sleep is the deepest stage of sleep, i.e., it is the stage with the highest threshold for waking up. In an attempt to explain this paradox, it was shown, using magnetoencephalography in humans, that the main difference in responsiveness during the two states is their effect on the ongoing gamma (~40 Hz) oscillations (*28*). Responses to auditory clicks caused a reset of the ongoing gamma rhythm, whereas during REM, the evoked response did not change the phase of the ongoing oscillation; these find-

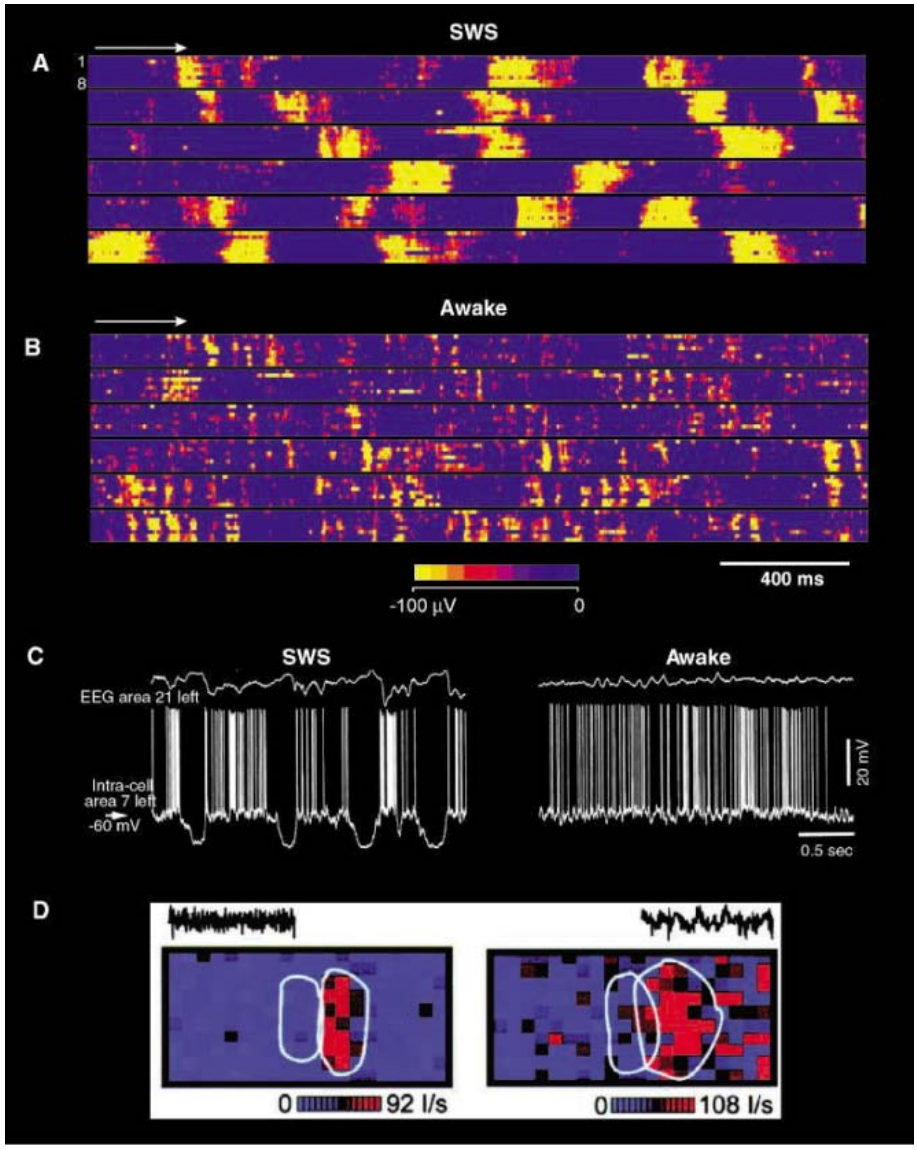


Fig. 1. Complex spatiotemporal patterns of ongoing network activity during wake and sleep states in neocortex. (**A**) Spatiotemporal map of activity computed from multiple extracellular local field potential (LFP) recordings in a naturally sleeping cat during slow-wave sleep (SWS). The activity consists of highly synchronized slow waves (in the δ frequency range, 1–4 Hz), which are irregular temporally but coherent spatially. (**B**) Same recording arrangement when the animal was awake. In this case, the β frequency–dominated LFPs (15–30 Hz) are weakly synchronized and very irregular both spatially and temporally. [(A) and (B) modified from (*73*)] (**C**) Intracellular recordings during these two states show slow oscillations during slow-wave sleep (SWS, left), and a sustained depolarized state with intense fluctuations during wakefulness (Awake, right). [Courtesy of Igor Timofeev, Laval University] (**D**) Network state–dependent responsiveness in visual cortex. Cortical receptive fields obtained by reverse correlation in simple cells for ON responses. The procedure was repeated for different cortical states, by varying the depth of the anesthesia (EEG indicated above each color map). (Left) Desynchronized EEG states (light anesthesia); (right) synchronized EEG states with prominent slow oscillatory components (deeper anesthesia). Receptive fields were always smaller during desynchronized states. Color code for spike rate (see scale). [Modified from (*12*)]

ings suggest that, during dream sleep, sensory input is not incorporated into the context represented by the ongoing activity (*29*). The obvious conclusion is that much smaller changes in network dynamics than those that differentiate sleep and waking are critical in determining the processing state of the brain. The failure to detect the differences in network dynamics that must exist between waking and REM sleep is a clear indication that new approaches are necessary.

Another outstanding example of the role of intrinsic network dynamics in determining neuronal responsiveness is the effect of attention. Even though the parameters of network activity measured with current techniques seem to remain stable, shifts in attentional focus both in space (*30*) and time (*31*) increase the ability of the network to process stimuli by increasing neuronal sensitivity to stimuli. The neuronal mechanisms underlying attentional shifts are unknown; however, the effect of directed attention enhancing neuronal responsiveness and selectivity, as well as behavioral performance (*32*), is a clear indication of the critical role played by subtle changes of network dynamics in determining the outcome of network operations.

Two types of computer models discussed in the second part of this review attempt to capture the relation between network dynamics and neuronal responsiveness. Both classes of models explore the complex interaction between sensory inputs and noisy network states. In the first category of models, noise is generated externally and does not explicitly represent the properties of the network itself. In the second class of models, noise is generated intrinsically and is, therefore, constrained by the properties of the network, a state that is much closer to the in vivo situation.

The reverse problem is also of critical importance: how much the network dynamics are modified by ongoing sensory inputs. Although cortical and thalamic networks may be strongly activated by specific patterns of stimuli (*20*, *33*), such effects are likely due to the engagement of brainstem neuromodulatory systems, which receive dense collaterals from ascending sensory inputs (*5*). Recordings from visual cortex of awake, freely viewing ferrets (*34*) revealed that the spatial and temporal correlation between cells while natural scenes were viewed varies little when compared with values obtained during eyes closed. This subtle variation indicates that most of the spatial and temporal coordination of neuronal firing is driven by network activity and not by the complex visual stimulus. This paradigm is captured by network models in which the input is interrelated with the network state (see below).

In conclusion, the parameters that determine network dynamics have a critical effect in determining responsiveness and information representation. Network dynamics are likely to be defined at the single-cell level and, therefore, to elude current recording methods that either grossly undersample the population, such as multiunit recordings, or that average out neuronal specificity, such as field potentials or optical recordings. Therefore, critical transitions of network state underlying changes in responsiveness would go undetected by the global measures of activity currently in use. This underlines the important role of neuronal modeling to explore the properties of network dynamics in the irregular and noisy conditions of the waking state.

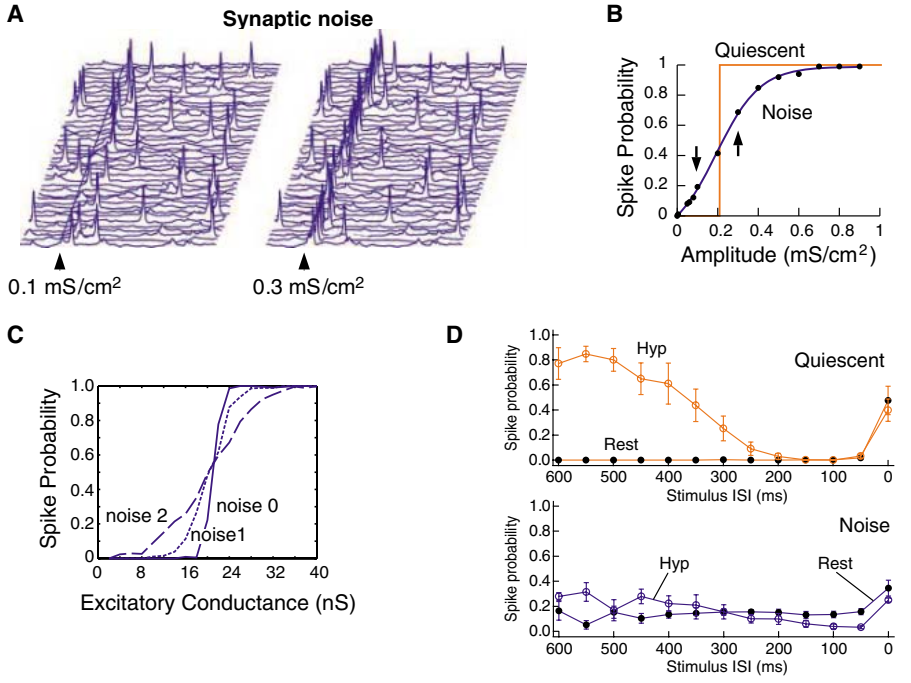


Fig. 2. Impact of network activity ("synaptic noise") on single neurons. **(A)** Single-trial responses of a model cortical neuron receiving synaptic noise. A stimulus (glutamatergic conductance) was delivered (arrow), either subthreshold (left) or suprathreshold (right). A fraction of the subthreshold stimuli gave rise to action potentials (left); however, not all suprathreshold stimuli gave a response. **(B)** Response curve computed from simulations similar to (A). The response curve gives the probability of action potential evoked by the stimulus, as a function of stimulus strength. In quiescent conditions, the response curve is all-or-none (action potential threshold around 0.2 mS/cm^2). With synaptic noise, subthreshold stimuli were boosted (downward arrow), while suprathreshold stimuli were attenuated (upward arrow). [(A) and (B) modified from (*42*)] **(C)** Effect of the amount of synaptic noise (measured by its variance; increasing noise levels from 0 to 2) on the response curve in real cortical neurons where synaptic noise was injected under dynamic clamp. [Modified from (*38*)] **(D)** Effect of synaptic noise on thalamic neurons. (Top) Spike probability as a function of interstimulus interval in a quiescent thalamic neuron stimulated by random glutamatergic conductances. The responsiveness was very different at hyperpolarized potentials (Hyp) because of the boosting effect of calcium currents and bursts. (Bottom) Same paradigm in the presence of synaptic noise. Here, the spike probability was nearly independent of stimulus ISI and of membrane potential. [Modified from (*44*)]

Computational Models of Network State–Dependent Computations

In the simplest type of computational model, the role of intrinsic network dynamics was investigated by representing irregular network activity as "noise" added to either single neurons or networks. Here, a variable presynaptic discharge, summed over many synapses, is approximated by "noise" imposed on the cell. A second, more elaborate type of model is to consider the state of the network explicitly and how network states can be used for various forms of computation. In the most sophisticated type of model, the input and network state are interdependent. We consider these three types of approaches successively.

Single-neuron and network responsiveness in the presence of noise. The simplest type of activity-dependent model is designed to consider the responsiveness of single neurons or networks in the presence of variable amounts of noise. Contrary to intuition, noise can have beneficial effects, especially in nonlinear systems driven by weak inputs. Such a positive effect of noise was first investigated by physicists and globally termed "stochastic resonance" (*35*), in which the signal-to-noise ratio is maximal for a nonzero level of noise. This type of paradigm is

relevant for the central nervous system and, in particular, for the cerebral cortex for several reasons. One is the nonlinearity of neurons, and another is that cortical neurons operate in vivo during the waking state in highly irregular or noisy states. Electrophysiological and modeling studies have measured the impact of cortical network activity during activated states on single neurons (through intracellular recordings). In parallel, models have estimated the conditions of "synaptic bombardment" that correspond to these measurements (*36*). These studies concluded that cortical neurons are in a "high-conductance state," in which synaptic activity causes large V_m fluctuations (also called synaptic noise) and an intense overall membrane conductance compared with the resting (leak) conductance of the neuron. Therefore, synaptic noise can have substantial effects on the behavior of the neuron. Despite this noisy aspect, high-conductance states provide computational advantages to neurons (*36*); the responsiveness to small inputs is enhanced by synaptic noise (Fig. 2, A and B), and the effect of synaptic inputs can become roughly independent of their location in dendrites (*37*). These effects are due to both the high conductance and the level of noise. Moreover, the small membrane time constant due to high conductances gives the neuron a better temporal resolution. Enhanced responsiveness can also be viewed as gain modulation and was also identified in real neurons by injecting artificial synaptic noise like that experienced in vivo by using dynamic clamp techniques (*38*–*42*). The response curve in the presence of noise is smooth (Fig. 2, B and C), so that subthreshold inputs are boosted, while suprathreshold inputs are attenuated (*43*) (arrows in Fig. 2B). Similar response curves were also obtained during the depolarizing phase of the slow oscillation in vitro (*38*). Synaptic noise can also combine with intrinsic properties, such as the low-threshold calcium currents in thalamic neurons, which lead to a responsiveness that is much less dependent on the V_m level (*44*) (Fig. 2D). This shows that intrinsic neuronal properties are expressed differently when considered together with network activity; both combine to yield a global responsiveness that depends on the properties of intrinsic currents and the amount of synaptic noise. Thus, network activity has a decisive impact on the input-output transformations of single neurons and confers to networks' fundamentally different information-processing capabilities as a function of their state.

Further evidence that network state has impacts on information processing comes from studies of the effect of noise in neural network models. Noise is beneficial to associative memories by avoiding convergence to spurious states (*45*); it enables networks to follow high-frequency stimuli (*46*), boosts the propagation of waves of activity (*47*), enhances input detection abilities (*48*, *49*), and enables populations of neurons to respond more rapidly (*50*–*52*). Noisy networks can also sustain a faithful propagation of firing rates [(*53*, *54*), but see (*55*)] or pulse packets (*56*) across successive layers (Fig. 3). The latter results are particularly interesting, because noise allows populations of neurons to relay a signal across successive layers without attenuation [in the case of firing rate propagation (Fig. 3C)] or prevents a catastrophic invasion of synchronous activity (Fig. 3D). The fact that a complex waveform propagates in a noisy network (Fig. 3C), but not with low noise levels (Fig. 3B), can be understood qualitatively from the

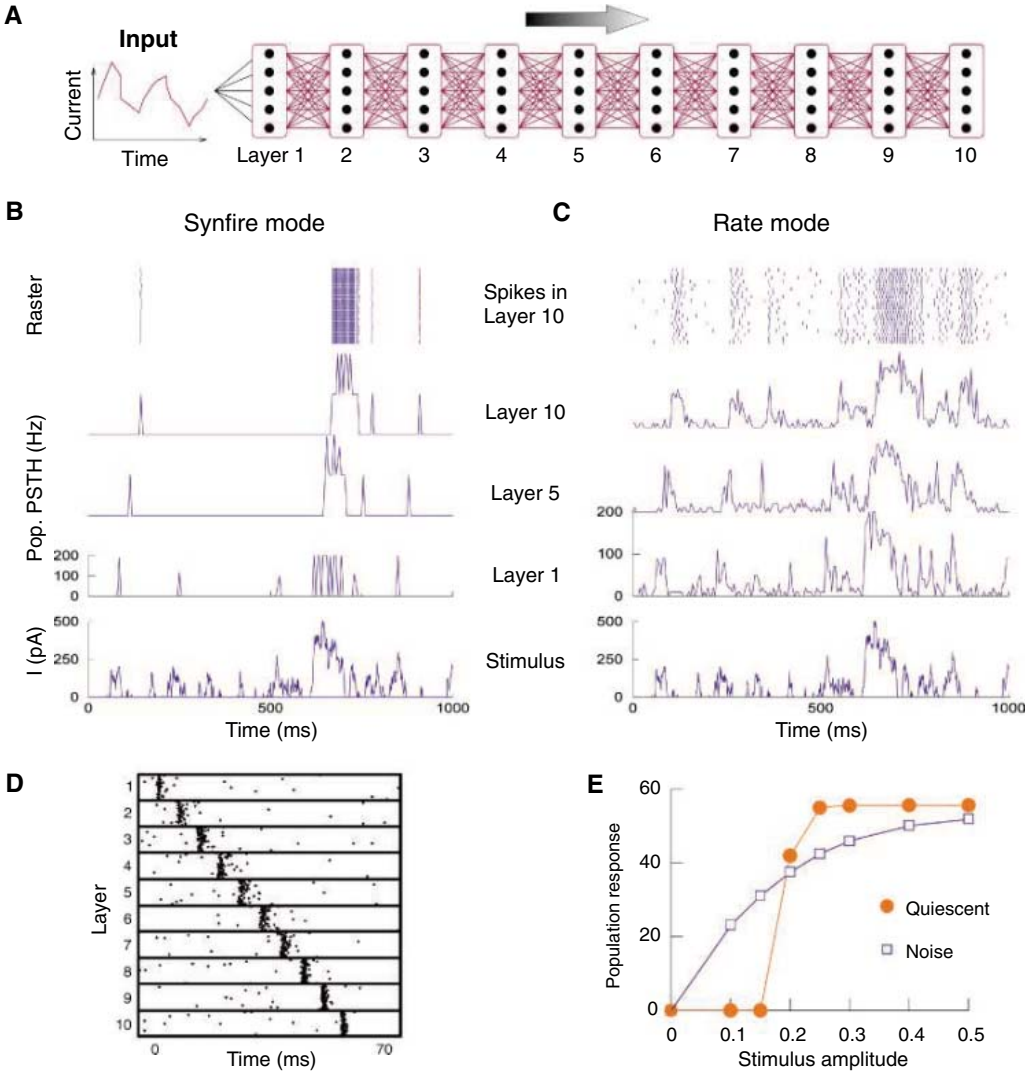


Fig. 3. Beneficial effects of noise at the network level. (**A**) Scheme of a multilayered network of integrate-and-fire (IF) neurons where layer 1 received a temporally varying input. (**B**) With low levels of noise ("synfire mode"), firing was only evoked for the strongest stimuli, and synchronous spike volleys propagated across the network. (**C**) With higher levels of noise ("Rate mode"), the network was able to reliably encode the stimulus and to propagate it across successive layers. [(A) to (C) modified from (*53*)] (**D**) Another example of a network able to sustain the propagation of synchronous volleys of spikes ("synfire chains") only in the presence of noise. [Modified from (*56*)] (**E**) Example of population response in a network of noisy neurons (noise), compared with the same network in the absence of noise (quiescent). Network response was close to all-or-none in quiescent conditions, but with noise, the population encoded stimulus amplitude more reliably. [Modified from (*42*)]

response curve of neurons in the presence of noise (Fig. 2B), for which there is a reliable coding of stimulus amplitude. Indeed, a similar effect is visible in the population response of networks of noisy neurons (Fig. 3E). With low noise levels, the nearly all–or–none response acts as a filter, which allows only strong stimuli to propagate and leads to propagation of synfire waves (Fig. 3D). With stronger noise levels, comparable to intracellular measurements in vivo, the response curve is progressive, which allows a large range of input amplitudes to be processed (Fig. 3C).

Thus, as for the single-cell paradigms discussed above, noise can have beneficial effects at the network level. Here also, noise can be thought of as representing the background network activity presynaptic to single cells, so these studies can be viewed as investigating network computations in states of irregular network activity. However, instead of explicitly modeling these states as generated by the network itself (see below), the study is performed in a quiescent network subject to external sources of noise. In this case, the main finding is that the nature of propagation of activity is fundamentally different—and in many cases, better—in the presence of noise.

Computing with intrinsic network states. A more elaborate type of model comes from explicitly considering the state of the network and its effect on computations or responsiveness to external inputs. Here again, one may find inspiration from physics, in particular from studies of how different dynamic states of matter provide different properties with respect to interactions with the environment. For example, in fluid dynamics, a fluid can adopt laminar or turbulent states when subject to different constraints. Turbulent states have considerably larger effective transport coefficients that enable the fluid to satisfy those constraints (*57*). A similar paradigm was applied to describe propagating activity in networks of excitatory and inhibitory neurons that display either silent, oscillatory (periodic), or irregular (chaotic or intermittent) states of activity (*58*) (Fig. 4A). Irregular states are optimal with respect to information transport [as defined by the diffusion coefficient for Shannon mutual information (Fig. 4A, right)]. Thus, similar to turbulence in fluids, irregular cortical states may represent a dynamic state that provides an optimal capacity for information transport in neural circuits. However, such an analogy must be refined by using more realistic models and connectivity (*59*).

More recent studies have explicitly considered networks endowed with intrinsically generated irregular activity states (*51*, *52*, *60*, *61*). Can the effect of noise on propagation discussed above be obtained when this noise is internally generated by the network? Such "internally generated noise," stemming from self-sustained irregular states of activity, was tested with respect to enhancing propagation properties in networks of excitatory and inhibitory neurons (*60*, *61*). However, propagation was difficult to observe; firing rates did not propagate unless synapses were reinforced (more than 10-fold) along specific feedforward pathways (*61*) (Fig. 4B). Similarly, pulse packets led to explosions of activity ("synfire explosions") in the network (Fig. 4C), and to enable propagation, synfire chains also had to be pre-embedded into the connectivity (*60*). Such embedding of feedforward pathways is of course not satisfactory, and the problem of how to obtain reliable propagation in recurrent networks is still open. The network states studied may not have the right level of excitability. In agreement with this, for firing rate models (*61*), a simple calculation shows that the total synaptic conductance in single cells is about 15 to 30 times as large as the leak conductance, which is about 5 times as large as in vivo measurements (*62*) and probably exerts an excessive shunting effect and counteracts propagation. Future studies should verify if better propagation capabilities are present in networks with a diversity of conductance states and cellular properties compatible with in vivo measurements, although such states may not be easy to obtain (*63*).

Interrelated input and network state. A further step in complexity corresponds to models where the input and the network state are interdependent. The simplest of such models is when external inputs influence network activity. Network activity will necessarily be influenced by external inputs, so the complex spatiotemporal activity in a given network is likely to reflect properties of the inputs and cannot be considered as independent. The first approach to take into account such dependence is the "liquid-state machine" paradigm (*64*). In this case, a network of spiking neurons is maintained in a self-sustained irregular state, and the network receives ongoing inputs. A few cells from the network are taken as output, and their

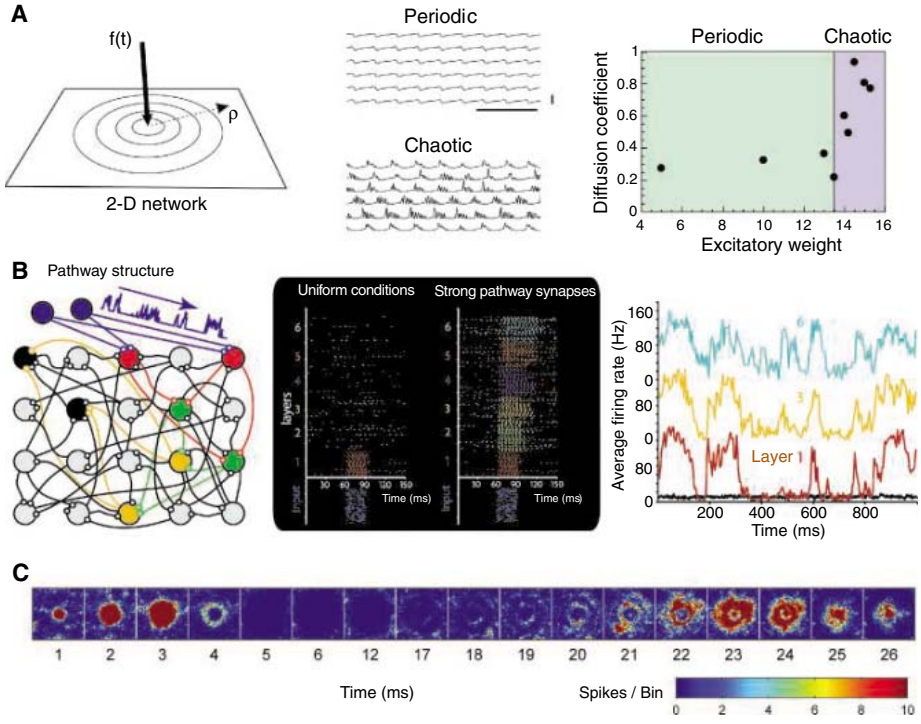


Fig. 4. Role of internally generated noise on information propagation in networks. (**A**) (Left) Stimulation paradigm consisting of injecting a complex waveform [*f*(*t*), left] and monitoring the spread of activity as a function of distance (ρ) and state of the network. (Middle) Example of two self-sustained dynamic states of the network, periodic oscillations (top) and irregular activity ("chaotic," bottom). (Right) Diffusion coefficient calculated for Shannon information as a function of the state of the network. Periodic states (green) had a relatively low diffusion coefficient, whereas, for irregular or chaotic states (blue), information transport was enhanced. [Modified from (*58*)] (**B**) Propagation of activity in a network of neurons displaying self-sustained irregular states. (Left) Definition of successive layers and pathways; (middle) absence of propagation with uniform conditions (left) contrasted with propagation when pathway synapses were reinforced (right); (right) propagation of a time-varying stimulus with pathway synapses reinforced. [Modified from (*61*)] (**C**) Propagation of activity in a network with self-sustained irregular dynamics. Successive snapshots illustrate that a stimulus (leftmost, red) led to an "explosion" of activity, followed by silence and echoes. [Modified from (*60*)]

noisy pattern of activity is decoded by "readout units," which can learn to extract information about the inputs. The activity of neurons at a given time contains information about the input at prior times, thus it is possible to generate a desired output at any time in a system receiving ongoing inputs. Information is stored in the activity of the network, which necessarily reflects input history. Thus, in the liquid-state machine paradigm, network activity acts as a sophisticated nonlinear filter, and although different network states were not explored, this approach has the merit of proposing a computing paradigm that explicitly uses complex dynamics in network activity as a means to integrate information [see also (*65*, *66*)]. This type of paradigm is also compatible with results showing that cortical sensory responses primarily reflect modulations of network activity rather than being input-driven (*34*).

Finally, the most sophisticated paradigm is that in which the input itself depends on network state. This type of modulation has been found in several sensory systems where internally generated signals are matched with sensory inputs. This is the case for the corollary discharge (also termed efference copy), which represents a copy of the internally generated motor command, which is matched to sensory inputs, performing cancellation or prediction (*67*–*70*). A similar interaction may arise more generally through thalamocortical loops; the cortex massively projects to the same thalamic cells from which cortical input originates, and cortical synapses on thalamic neurons outnumber peripheral synapses by about one order of magnitude (*71*). Such a massive corticothalamic feedback can potentially modulate, complement, or even predict sensory inputs. In these cases, cortical network state will necessarily influence and modulate its own inputs (*72*). Such bidirectional interactions between input and network state are, of course, considerably more difficult to model and constitute clear challenges for future studies.

Conclusion

Much remains to be done to properly characterize internal brain dynamics and how they modulate computations. We need to obtain adequate experimental methods to properly measure the different dynamic states exhibited by neural circuits, and how network activity is modulated by parameters such as attention or sensory inputs. To characterize their computational properties, modeling studies have so far implicitly assumed that a given network produces only one prototypical state of irregular activity, but evidence indicates that this may not be true in general [in Fig. 3A (right), for example, the information flow can be up to two times larger between different chaotic states (*58*)]. Furthermore, networks may switch rapidly among states according to rules not yet known (*33*) and with important consequences for information processing. One approach would be to characterize intracellularly the diverse dynamics of fluctuations (or oscillations) in single cells and to model their effect on the neuron's input/output function. This single-cell characterization can then be used to infer propagating properties at the network level, constrained by global recordings methods such as imaging. It is only through a tight combination of experiments and models that we will better understand the computational properties of internally generated brain states.

References and Notes

1. R. R. Llinas, *Science* **242**, 1654 (1988).
2. M. Steriade, *Neuroscience* **101**, 243 (2000).
3. D. A. McCormick, *Prog. Neurobiol.* **39**, 337 (1992).
4. M. Steriade, D. A. McCormick, T. J. Sejnowski, *Science* **262**, 679 (1993).
5. M. Steriade, *Neuronal Substrates of Sleep and Epilepsy* (Cambridge Univ. Press, Cambridge, 2003).
6. D. Contreras, M. Steriade, *J. Neurosci.* **15**, 604 (1995).
7. M. Steriade, I. Timofeev, F. Grenier, *J. Neurophysiol.* **85**, 1969 (2001).
8. M. Steriade, D. Contreras, F. Amzica, I. Timofeev, *J. Neurosci.* **16**, 2788 (1996).
9. I. Lampl, Y. Yarom, *J. Neurophysiol.* **70**, 2181 (1993).
10. W. Singer, *Neuron* **24**, 49 (1999).
11. M. S. Livingstone, D. H. Hubel, *Nature* **291**, 554 (1981).
12. F. Worgotter *et al.*, *Nature* **396**, 165 (1998).
13. B. Li, K. Funke, F. Worgotter, U. T. Eysel, *J. Physiol.* **514**, 857 (1999).
14. K. Funke, U. T. Eysel, *Brain Res.* **573**, 217 (1992).
15. A. Arieli, A. Sterkin, A. Grinvald, A. Aertsen, *Science* **273**, 1868 (1996).
16. M. Tsodyks, T. Kenet, A. Grinvald, A. Arieli, *Science* **286**, 1943 (1999).
17. T. J. Morrow, K. L. Casey, *J. Neurophysiol.* **67**, 305 (1992).
18. J. M. Edeline, G. Dutrieux, Y. Manunta, E. Hennevin, *Eur. J. Neurosci.* **14**, 1865 (2001).
19. M. A. Kisley, G. L. Gerstein, *J. Neurosci.* **19**, 10451 (1999).
20. L. M. Miller, C. E. Schreiner, *J. Neurosci.* **20**, 7011 (2000).
21. M. Murakami, H. Kashiwadani, Y. Kirino, K. Mori, *Neuron* **46**, 285 (2005).
22. M. Steriade, M. Deschenes, *Brain Res.* **320**, 1 (1984).
23. R. R. Llinas, M. Steriade, *J. Neurophysiol.* **95**, 3297 (2006).
24. D. Contreras, M. Steriade, *Neuroscience* **76**, 11 (1997).
25. M. Steriade, *Electroencephalogr. Clin. Neurophysiol.* **27**, 678 (1969).
26. M. Steriade, G. Iosif, V. Apostol, *J. Neurophysiol.* **32**, 251 (1969).
27. M. Steriade, M. Deschenes, G. Oakson, *J. Neurophysiol.* **37**, 1065 (1974).
28. R. Llinas, U. Ribary, *Proc. Natl. Acad. Sci. U.S.A.* **90**, 2078 (1993).
29. R. R. Llinas, D. Pare, *Neuroscience* **44**, 521 (1991).
30. C. E. Connor, D. C. Preddie, J. L. Gallant, D. C. Van Essen, *J. Neurosci.* **17**, 3201 (1997).
31. G. M. Ghose, J. H. Maunsell, *Nature* **419**, 616 (2002).
32. H. Spitzer, R. Desimone, J. Moran, *Science* **240**, 338 (1988).
33. Y. Fregnac, paper presented at *Cosyne: Computational and Systems Neuroscience 2006*, Salt Lake City, Utah, 5 to 8 March, 2006.
34. J. Fiser, C. Chiu, M. Weliky, *Nature* **431**, 573 (2004).
35. K. Wiesenfeld, F. Moss, *Nature* **373**, 33 (1995).
36. A. Destexhe, M. Rudolph, D. Pare, *Nat. Rev. Neurosci.* **4**, 739 (2003).
37. M. Rudolph, A. Destexhe, *J. Neurosci.* **23**, 2466 (2003).
38. Y. Shu, A. Hasenstaub, M. Badoual, T. Bal, D. A. McCormick, *J. Neurosci.* **23**, 10388 (2003).
39. F. S. Chance, L. F. Abbott, A. D. Reyes, *Neuron* **35**, 773 (2002).
40. A. Destexhe, M. Rudolph, J. M. Fellous, T. J. Sejnowski, *Neuroscience* **107**, 13 (2001).
41. J. M. Fellous, M. Rudolph, A. Destexhe, T. J. Sejnowski, *Neuroscience* **122**, 811 (2003).
42. D. Jaeger, J. M. Bower, *J. Neurosci.* **19**, 6090 (1999).
43. N. Ho, A. Destexhe, *J. Neurophysiol.* **84**, 1488 (2000).
44. J. Wolfart, D. Debay, G. Le Masson, A. Destexhe, T. Bal, *Nat. Neurosci.* **8**, 1760 (2005).
45. D. J. Amit, *Modeling Brain Function* (Cambridge Univ. Press, Cambridge, USA, 1989).
46. B. W. Knight, *J. Gen. Physiol.* **59**, 734 (1972).
47. P. Jung, G. Mayer-Kress, *Phys. Rev. Lett.* **74**, 2130 (1995).
48. J. J. Collins, C. C. Chow, T. T. Imhoff, *Nature* **376**, 236 (1995).
49. N. G. Stocks, R. Mannella, *Phys. Rev. E Stat. Nonlin. Soft Matter Phys.* **64**, 030902 (2001).
50. G. Silberberg, M. Bethge, H. Markram, K. Pawelzik, M. Tsodyks, *J. Neurophysiol.* **91**, 704 (2004).
51. M. Tsodyks, T. J. Sejnowski, *Netw. Computat. Neural Syst.* **6**, 111 (1995).
52. C. van Vreeswijk, H. Sompolinsky, *Science* **274**, 1724 (1996).
53. M. C. van Rossum, G. G. Turrigiano, S. B. Nelson, *J. Neurosci.* **22**, 1956 (2002).
54. A. D. Reyes, *Nat. Neurosci.* **6**, 593 (2003).
55. V. Litvak, H. Sompolinsky, I. Segev, M. Abeles, *J. Neurosci.* **23**, 3006 (2003).
56. M. Diesmann, M. O. Gewaltig, A. Aertsen, *Nature* **402**, 529 (1999).
57. S. B. Pope, *Turbulent Flows* (Cambridge Univ. Press, Cambridge, 2000).
58. A. Destexhe, *Phys. Rev. E Stat. Phys. Plasmas Fluids Relat. Interdiscip. Topics* **50**, 1594 (1994).
59. Preliminary results from S. El Boustani and A. Destexhe indicate that such an analogy also holds for more realistic connectivity schemes.
60. C. Mehring, U. Hehl, M. Kubo, M. Diesmann, A. Aertsen, *Biol. Cybern.* **88**, 395 (2003).
61. T. P. Vogels, L. F. Abbott, *J. Neurosci.* **25**, 10786 (2005).
62. D. Pare, E. Shink, H. Gaudreau, A. Destexhe, E. J. Lang, *J. Neurophysiol.* **79**, 1450 (1998).
63. F. Alvarez, A. Destexhe, *Neurocomputing* **58**, 285 (2004).
64. W. Maass, H. Markram, *Neural Netw.* **15**, 155 (2002).
65. D. V. Buonomano, M. M. Merzenich, *Science* **267**, 1028 (1995).
66. J. J. Hopfield, C. D. Brody, *Proc. Natl. Acad. Sci. U.S.A.* **98**, 1282 (2001).
67. M. A. Sommer, R. H. Wurtz, *Science* **296**, 1480 (2002).
68. R. W. Sperry, *J. Comp. Physiol. Psychol.* **43**, 482 (1950).
69. D. M. Wolpert, J. R. Flanagan, *Curr. Biol.* **11**, R729 (2001).
70. C. C. Bell, A. Caputi, K. Grant, J. Serrier, *Proc. Natl. Acad. Sci. U.S.A.* **90**, 4650 (1993).
71. M. Steriade, E. G. Jones, D. A. McCormick, *Thalamus* (Elsevier, Amsterdam, 1997).
72. A similar interaction could also arise from corticocortical loops: A given cortical area may modulate its own cortical inputs by directly influencing the state of activity in the area from which these inputs originate.
73. A. Destexhe, D. Contreras, M. Steriade, *J. Neurosci.* **19**, 4595 (1999).

74. We dedicate this article to Mircea Steriade. We thank S. El Boustani, L. Finkel, Y. Frégnac, and A. Herz for comments on the manuscript. We acknowledge support from NIH, the Human Frontier Science Program, the European Community, and the CNRS.

10.1126/science.1127241

REVIEW

Biologically Based Computational Models of High-Level Cognition

Randall C. O'Reilly

Computer models based on the detailed biology of the brain can help us understand the myriad complexities of human cognition and intelligence. Here, we review models of the higher level aspects of human intelligence, which depend critically on the prefrontal cortex and associated subcortical areas. The picture emerging from a convergence of detailed mechanistic models and more abstract functional models represents a synthesis between analog and digital forms of computation. Specifically, the need for robust active maintenance and rapid updating of information in the prefrontal cortex appears to be satisfied by bistable activation states and dynamic gating mechanisms. These mechanisms are fundamental to digital computers and may be critical for the distinctive aspects of human intelligence.

Biologically based computational modeling has been an integral part of many basic areas of cognitive neuroscience (e.g., perception and memory). More recently, these mechanistic approaches have been encroaching on some of the most mysterious and challenging higher level areas of human cognition, including decision making, problem solving, and "executive" control of cognition and action. From a biological perspective, it is clear that the prefrontal cortex (PFC) and associated subcortical areas in the basal ganglia and midbrain play a disproportionately important role in these aspects of cognition (*1*, *2*). Furthermore, the PFC is the area of cortex most greatly expanded in humans relative to other mammals (*3*), suggesting that it is critical to human intellectual abilities.

Two examples are instructive. First, people with damage to PFC areas often exhibit environmental dependency syndrome (*4*), which is (provocatively) just a fancy name for a lack of free will: Behavior is driven more by the external environment than by internal plans or goals. For example, one person with PFC damage visiting a researcher's home saw a bed and proceeded to get undressed (including removing his toupee), got into bed, and prepared to sleep. The second example is something to which everyone can relate: the crazy world of dreams. One minute you are talking with a long-forgotten friend from high school and the next you are late for an airplane you cannot quite seem to find. The PFC is one of the primary brain areas deactivated during dream states (*5*), and its absence may have much to do with the lack of temporal contiguity and inability to stay on task that are characteristic of dreams. In short, the PFC is critical for maintaining current context, goals, and other information in an active state that guides ongoing behavior in a coherent, task-relevant manner (*2*, *6*).

It is not enough to state simply that the PFC houses our internal "executive" that decides what we want to do and keeps us focused on those goals in the face of various environmental distractions. That only labels and locates the mystery. The promise of biologically based computational models is that they can actually break open these mysteries by describing the underlying mechanisms in precise computational detail and showing that they are indeed capable of the functions attributed to the PFC. The success of this approach does not mean that we need to think of humans as robots. Instead, these models show that many subtle factors interact in complex ways to produce the emergent phenomenon of cognitive control, which cannot be simply reduced to its constituents. The modeling approach enables such complex and subtle interactions to be understood in a way that would be impossible using the comparatively blunt empirical methods available today. The risk run by these models is that they provide an elaborate fiction, instead of facts, about how the brain actually works. However, this risk can be mitigated by building models that integrate a wide range of empirical data spanning many different levels of analysis.

Here, we focus on converging models at multiple levels of analysis that together paint a remarkably coherent picture of PFC and associated systems. We first review some areas of fundamental agreement and provide an initial sketch of this emerging picture, followed by some specific recent developments.

The Standard Model of Prefrontal Cortex Function: Active Maintenance, Top-Down Control, and Rapid Updating

The PFC is important for actively maintaining information by sustained neural firing (*7*, *8*), which is robust in the face of potentially distracting information [i.e., working memory (*9*)]. This is in contrast with other cortical areas, which tend to be swayed by whatever stimulus is currently impinging on them (hence the environmental dependency syndrome in the absence of normal PFC function). From this basic mechanism of active maintenance, it is possible to explain a remarkable amount of what the PFC does. For example, the robust active maintenance of a goal or plan representation (e.g., "go to the grocery store before going home") can guide a sequence of behaviors (e.g., making the appropriate turns) simply by providing additional neural activation to these appropriate behaviors in the face of other possibly stronger competing actions (e.g., driving directly home). Because such goals can be maintained in the face of inevitable environmental distractors, they enable behavior to be consistent and coherent over time. Accordingly, when PFC is not functioning well, as in the dream state or in the prevalent attention deficit hyperactivity disorder (ADHD) (*10*, *11*), behavior becomes less consistent and coherent over time. Furthermore, because of this ability to focus on a task to the exclusion of other distracting information, the PFC is often characterized as inhibiting task-irrelevant information (*12*, *13*).

The PFC system is also capable of rapidly updating what is being maintained, which is critical for behavioral flexibility—the ability to quickly adapt to the changing demands of the environment. People with PFC damage tend to perseverate in the face of changing task demands (*14*), as do young children with immature PFC function (*15*). Areas within PFC also play a key role in monitoring of behavior [necessary for applying appropriate levels of control, e.g., (*16*)] and in emotional and reward processing (*17*). These are beyond the scope of the present paper, but it may be possible to understand many aspects of these functions using the basic mechanisms elaborated below (*18*).

We return to our main question: How does the brain actually perform these active maintenance and rapid updating functions in terms of detailed biological mechanisms? Biologically based computational models have explored this question in depth. The emerging picture can be summarized in terms of analog versus digital computation; whereas the rest of cortex can be characterized as a fundamentally analog system operating on graded, distributed information, the prefrontal cortex has a more discrete, digital character. Robust active maintenance is supported by a form of bistability, which means that neurons switch between two stable states (off or on), much as bits in a computer. Rapid updating requires a mechanism for gating or switching between these bistable states—this gating/switching is the essential function of a transistor in a digital

Department of Psychology, University of Colorado Boulder, Boulder, CO 80309, USA. E-mail: oreilly@psych.colorado.edu

computer. Even though each of these mechanisms is strongly motivated from basic biological and computational factors having nothing to do with digital computation, the parallels are striking and might perhaps provide some critical insight into what makes humans distinctively intelligent.

Biological Mechanisms of Active Maintenance and Rapid Updating: Bistability and Gating

Perhaps the most obvious mechanism for active maintenance is recurrent excitatory connectivity, which amounts to a form of you-rub-my-back-and-I'll-rub-yours. Active neurons send excitation to other neurons that then send excitation back, creating a stable "attractor" state (*19*). Although appealingly simple, several problems with this model have arisen. For example, biologically detailed models have shown that it is difficult to sustain this positive feedback system because individual spikes of neural firing may not come frequently enough to keep it going (*20*). Furthermore, when using these attractor states to integrate information over time, it has become clear that noise (which is ubiquitous in the brain) quickly swamps any signal present in these systems (*21*). Intuitively, the system operates like the classic "telephone" game, where a message passed continuously along a chain (or repeatedly among an interconnected population of neurons) is rapidly distorted.

A solution to both of the above problems is to incorporate some form of intrinsic bistability into the neural systems (*20*–*22*). In these models, bistability comes from gated ion channels that require specific levels of neural depolarization to be activated, and once active they remain so for hundreds of milliseconds or more [e.g., the *N*-methyl-D-aspartate (NMDA) channel]. This imparts a critical degree of robustness to the active maintenance abilities of PFC neurons, enabling them to span the gaps between spikes and also not to get blown around by the winds of neural noise (*21*). To encode analog (graded) information with bistable neurons, the system must use distributed binary representations that work somewhat like a binary encoding of a floating-point number on a computer: Many neurons (bits) work together such that the combined pattern of activity represents different values. Although this is less efficient than a direct analog representation (which could be done with a single neuron), the improvement in robustness may be worth this cost. Certainly, this is the case with computers. We use digital computers because analog computers are quickly swamped by noise.

Rapid updating provides another important challenge for neural mechanisms to solve, because it directly conflicts with the need for robust active maintenance. Once a set of neurons is locked into a stable state, how can it subsequently be updated to hold on to new information? A number of mechanistic solutions to this problem have been proposed, all of which amount to a dynamic gating mechanism, which modulates the stability of PFC active maintenance. When the gate is open, PFC is rapidly updated with new information. When the gate is closed, it robustly maintains existing information.

One class of gating mechanisms depends on the neuromodulator dopamine (*22*–*25*), which is transmitted to the PFC by the midbrain ventral tegmental area (VTA). In all such models, dynamic changes in the level of dopamine in PFC, caused by phasic VTA firing above the normal tonic level, switch the system between rapid updating and robust maintenance (Fig. 1). Biologically detailed computational modeling has made sense of the dense and confusing thicket of studies on dopamine modulation of PFC circuits (*22*, *25*). In this model, dopamine D1 receptor activation produces a net overall effect of stabilizing working memory states in PFC through a complex combination of seemingly opposing effects, including increased NMDA current activation (*20*). In contrast, D2 receptor activation produces opposing destabilizing effects. Given that both receptors are activated by the same neuromodulator, how does the system alternate between maintenance and updating? D2 receptors are largely synaptic and respond only to high concentrations of dopamine, whereas D1 receptors are extrasynaptic and respond to lower concentrations. Thus, a phasic burst of dopamine will activate D2 receptors located in the synapses and trigger rapid updating, whereas lower tonic concentrations diffusing extrasynaptically produce a default level of robust maintenance (*25*) (Fig. 1). This biologically detailed model converges remarkably well with earlier, more abstract computational models hypothesizing that dopamine modulates the gain or signal-to-noise ratio of neurons (*24*, *26*).

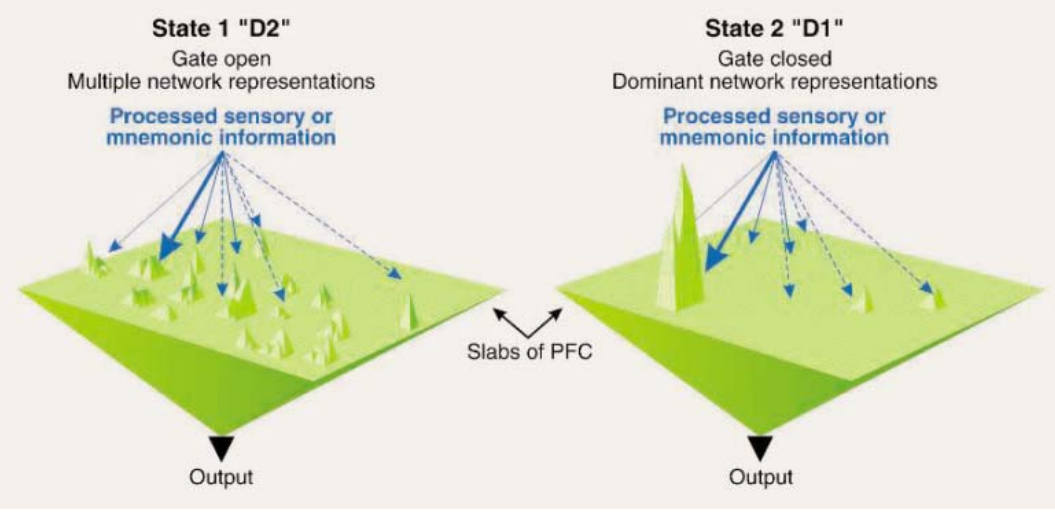


Fig. 1. Dopamine-based gating mechanism that emerges from the detailed biological model of Durstewitz, Seamans, and colleagues (*22*, *25*). The opening of the gate occurs in the dopamine D2-receptor–dominated state (State 1), in which any existing active maintenance is destabilized and the system is more responsive to inputs. The closing of the gate occurs in the D1-receptor–dominated state (State 2), which stabilizes the strongest activation pattern for robust active maintenance. D2 receptors are located synaptically and require high concentrations of dopamine and are therefore activated only during phasic dopamine bursts, which thus trigger rapid updating. D1 receptors are extrasynaptic and respond to lower concentrations, so robust maintenance is the default state of the system with normal tonic levels of dopamine firing.

Another class of gating mechanism leverages the extensive connectivity between the PFC and the basal ganglia (*27*–*29*) (Fig. 2). Direct pathway "Go" neurons in the basal ganglia can trigger a phasic wave of activation into PFC through a modulatory disinhibition effect (Fig. 2), which results in rapid updating of PFC states. These Go neurons are opposed by a set of indirect pathway "NoGo" neurons that prevent this phasic wave of activation and enable the default state of robust active maintenance to continue. This basal ganglia mechanism is functionally very similar to the dopamine-based one. However, a key difference is that the basal ganglia mechanism enables selective gating of only some regions of PFC, whereas dopamine modulation is more broad and diffuse. Furthermore,

the dopaminergic effects in PFC are thought to operate on a slower time scale than the basal ganglia (*25*). Thus, it is likely that the basal ganglia gating supports more rapid, selective updating of specific regions in PFC, whereas the dopaminergic gating provides a longer time scale, broader gating signal that is modulated by overall performance levels.

The presence of a gating mechanism raises the question of how the gate knows when to open and close. Several models have shown that reinforcement learning mechanisms, which also involve the dopaminergic system (synergistically with its role in gating), can learn to control this gating mechanism (*23*, *29*). One of these models was shown to compare favorably with some of the most advanced but biologically implausible learning mechanisms on complex temporally extended working memory tasks (*29*). This model is now being tested on a wide range of benchmark higher level cognition tasks to determine whether the biologically based mechanisms converge with behavioral data (*30*). Thus, it is plausible, but not yet established, that such a system could learn to perform the many different higher level cognitive tasks that humans can perform.

Toward Higher Digital Intelligence

The "digital" picture emerging from these bistable, dynamically gated PFC neurons supports a certain amount of intelligent behavior, at least in the terms that were considered above: robust active maintenance of goals and other task-relevant information, and rapid updating of this information to keep pace with a changing environment or task. Is that the full story, or are there other important ingredients to our intelligence?

These digital PFC dynamics may also support more abstract forms of reasoning, which is another important aspect of human intelligence (*31*). The presence of a dynamic gating mechanism and robust active maintenance in the PFC led to the development of more abstract, rulelike representations in a simulated PFC. Although these representations do not have the arbitrary flexibility of the symbols present in digital computer programs (and symbolic models of human cognition), they are nevertheless closer than the graded, distributed representations typically associated with other areas of cortex. Specifically, these PFC representations in the model enabled the behavior of the overall system to be more regular (i.e., describable by an abstract rule), in that it could more consistently apply a previously learned rule to novel situations. This model is consistent with recent recordings from PFC and posterior cortex neurons in monkeys, which showed that PFC neurons exhibit more abstract rulelike encodings of categories and other task-relevant information (*32*–*34*).

The fact that monkeys also show some degree of abstract representation in PFC raises the perennial question of what exactly is different between us and them. The critical difference may be that people have a basic social instinct for sharing experiences and knowledge with each other that is largely absent in even our closest primate relatives (*35*). Thus, the qualitative difference comes not from the hardware [which is still quantitatively better (*3*)] but from the motivations that drive us to spend so much time learning and communicating what we have learned to others. This account dovetails nicely with the above modeling work (*31*), which found that the abstract PFC representations took a long time to develop and required integrating knowledge across multiple different but related tasks. Furthermore, the development of the PFC is the most protracted of any brain area, not fully maturing until adolescence (*15*, *36*, *37*). Thus, the full glory of human intelligence requires the extensive, culturally supported developmental learning process that takes place over the first decades of life.

Another potential implication of a dynamic gating mechanism is the ability to perform transistor-like dynamic switching, which can enable a form of variable binding (i.e., assigning arbitrary information to a given functional role, as in "let $X = 7$") that is not otherwise possible in statically connected neural circuits (*29*, *38*). Figure 3 provides a simple illustration of this form of variable binding, in which one input signal (a verb in a sentence) can dynamically control (through the basal ganglia) which of two different PFC representations encode the name of a person. If the verb form is active, then the name is encoded in the PFC neurons that represent the agent (actor) of the sentence; if the verb is passive, then the patient (object) representations are activated. This system can be more flexible than other more static neural circuits because the gating signal can be completely independent of the content that is being gated. However, unlike a memory buffer in a standard digital computer, the PFC areas must learn slowly over time to be able to represent all the things that they can maintain, and other areas of the brain must similarly learn to decode both the content and role meaning of these PFC representations. Thus, the dynamic variable binding operates in the context of the relatively more static learned

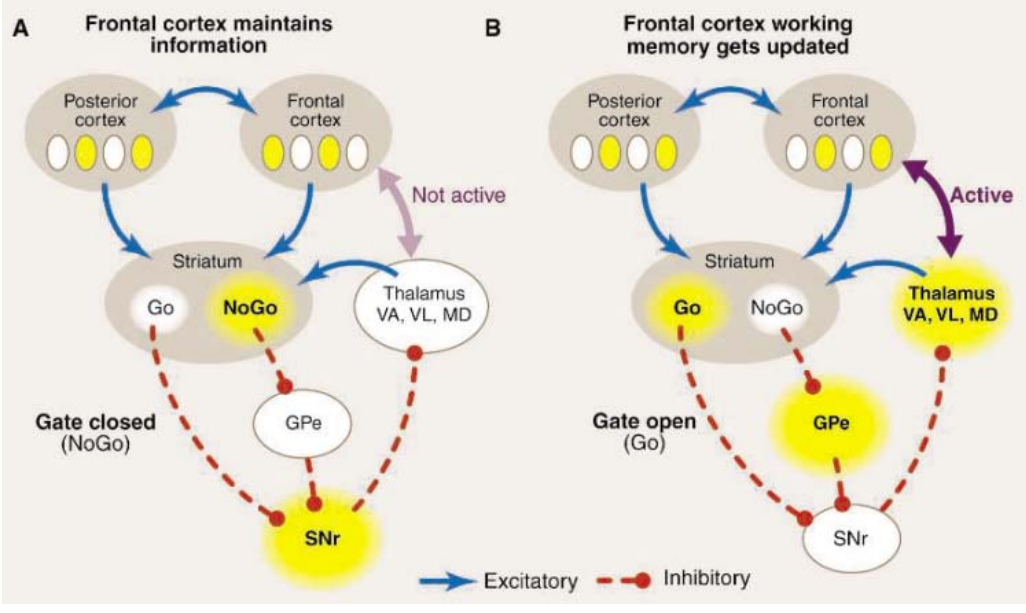


Fig. 2. Dynamic gating produced by disinhibitory circuits through the basal ganglia and frontal cortex/PFC (one of multiple parallel circuits shown). (**A**) In the base state (no striatum activity) and when NoGo (indirect pathway) striatum neurons are firing more than Go, the SNr (substantia nigra pars reticulata) is tonically active and inhibits excitatory loops through the basal ganglia and PFC through the thalamus. This corresponds to the gate being closed, and PFC continues to robustly maintain ongoing activity (which does not match the activity pattern in the posterior cortex, as indicated). (**B**) When direct pathway Go neurons in striatum fire, they inhibit the SNr and thus disinhibit the excitatory loops through the thalamus and the frontal cortex, producing a gating-like modulation that triggers the update of working memory representations in prefrontal cortex. This corresponds to the gate being open.

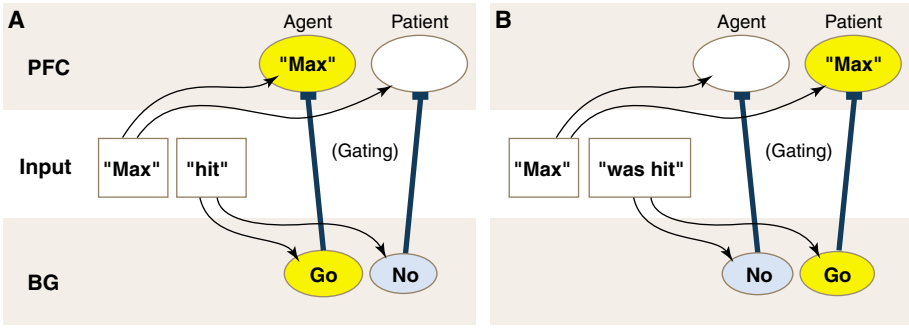


Fig. 3. Dynamic gating can achieve a form of dynamic variable binding, illustrated here for assigning the semantic role of a person based on the grammatical structure of incoming text. The basal ganglia (BG) provides dynamic gating signals to different PFC areas that have learned to encode either the Agent (actor) or Patient (object) semantic role information. If a given set of BG neurons fire a Go (update) signal, then current sensory information is updated into corresponding PFC neurons; if the corresponding BG neurons have a NoGo (do not update) signal, the PFC area is not updated. In (**A**), the active form of the verb ("hit") causes the BG gating units to fire a "Go" (update) signal for the Agent role representations in PFC, which then represent the incoming name ("Max"). The Patient role is not updated because of a NoGo (do not update) signal. In (**B**), the passive form ("was hit") activates the opposite pattern of BG gating, resulting in "Max" being encoded in the Patient role. This ability for one signal (the verb in this case) to modulate where another piece of information ("Max") is encoded provides a basic form of variable binding.

structures typical of other cortical areas and therefore does not achieve the completely arbitrary character of a digital computer. This constraint has benefits, however, because the PFC neurons automatically have meaning through their learned connections with other neurons, and this "grounds" what would otherwise be arbitrary, meaningless symbols.

Having a biologically based mechanism for limited variable binding opens up new opportunities to develop links between these models and more abstract cognitive architectures such as ACT-R (adaptive control of thought–rational) (*39*) that can actually perform complex problem solving and other higher level cognitive tasks that are beyond the reach of existing biologically detailed models. A highly constrained form of variable binding is critical for most cognitive operations in ACT-R, and there is some recent indication that a somewhat more flexible form of binding ("dynamic pattern matching") is necessary for distinctively human cognitive abilities (*40*). Establishing a clear neural basis for these properties would almost certainly provide important insights into what makes us so smart.

Conclusions

Scientists are always concerned about strongly differentiating theoretical positions: the long dominance and current disfavor of the computer metaphor for understanding the mind has led the new generation of biological neural network theorists to emphasize the graded, analog, distributed character of the brain. It is clear that the brain is much more like a social network than a digital computer, with learning, memory and processing all being performed locally through graded communication between interconnected neurons. These neurons build up strong, complex "relationships" over a long period of time; a neuron buried deep in the brain can only function by learning which of the other neurons it can trust to convey useful information. In contrast, a digital computer functions like the post office, routing arbitrary symbolic packages between passive memory structures through a centralized processing unit, without consideration for the contents of these packages. This affords arbitrary flexibility (any symbol is as good as any other), but at some cost: When everything is arbitrary, then it is difficult to encode the subtle and complex relationships present in our commonsense knowledge of the real world. In contrast, the highly social neural networks of the brain are great at keeping track of "who's who and what's what," but they lack flexibility, treating a new symbol like a stranger crashing the party.

The digital features of the PFC and associated areas help to broaden the horizons of naturally parochial neural networks. The dynamic gating mechanisms work more like a post-office, with the basal ganglia reading the zip code of which PFC stripe to update, whereas the PFC cares more about the contents of the package. Furthermore, the binary rulelike representations in the PFC are more symbol-like. Thus, perhaps a fuller understanding of this synthesis of analog and digital computation will finally unlock the mysteries of human intelligence.

References and Notes

1. J. M. Fuster, *The Prefrontal Cortex: Anatomy, Physiology and Neuropsychology of the Frontal Lobe* (Lippincott-Raven, New York, ed. 3, 1997).
2. E. K. Miller, J. D. Cohen, *Annu. Rev. Neurosci.* **24**, 167 (2001).
3. P. T. Schoenemann, M. J. Sheehan, L. D. Glotzer, *Nat. Neurosci.* **8**, 242 (2005).
4. F. Lhermitte, *Ann. Neurol.* **19**, 335 (1986).
5. J. A. Hobson, E. F. Pace-Schott, R. Stickgold, *Behav. Brain Sci.* **23**, 793 (2000).
6. R. C. O'Reilly, T. S. Braver, J. D. Cohen, *Models of Working Memory: Mechanisms of Active Maintenance and Executive Control*, A. Miyake, P. Shah, Eds. (Cambridge Univ. Press, New York, 1999), pp. 375–411.
7. J. M. Fuster, G. E. Alexander, *Science* **173**, 652 (1971).
8. P. S. Goldman-Rakic, *Handb. Physiol. Nervous Syst.* **5**, 373 (1987).
9. A. Miyake, P. Shah, Eds., *Models of Working Memory: Mechanisms of Active Maintenance and Executive Control* (Cambridge Univ. Press, New York, 1999).
10. A. L. Krain, F. X. Castellanos, *Clin. Psychol. Rev.* (2006).
11. E. G. Willcutt, A. E. Doyle, J. T. Nigg, S. V. Faraone, B. F. Pennington, *Biol. Psychiatry* **57**, 1336 (2005).
12. A. Diamond, *Dev. Sci.* **1**, 185 (1998).
13. J. M. Stedron, S. D. Sahni, Y. Munakata, *J. Cognit. Neurosci.* **17**, 623 (2005).
14. D. T. Stuss *et al.*, *Neuropsychologia* **38**, 388 (2000).
15. A. Diamond, P. S. Goldman-Rakic, *Exp. Brain Res.* **74**, 24 (1989).
16. M. Botvinick, L. E. Nystrom, K. Fissel, C. S. Carter, J. D. Cohen, *Nature* **402**, 179 (1999).
17. E. T. Rolls, *Cereb. Cortex* **10**, 284 (2000).
18. M. J. Frank, E. D. Claus, *Psychol. Rev.* **113**, 300 (2006).
19. D. Zipser, *Neural Comput.* **3**, 179 (1991).
20. X.-J. Wang, *J. Neurosci.* **19**, 9587 (1999).
21. A. A. Koulakov, S. Raghavachari, A. Kepecs, J. E. Lisman, *Nat. Neurosci.* **5**, 775 (2002).
22. D. Durstewitz, J. K. Seamans, T. J. Sejnowski, *J. Neurophysiol.* **83**, 1733 (2000).
23. T. S. Braver, J. D. Cohen, *Control of Cognitive Processes: Attention and Performance XVIII*, S. Monsell, J. Driver, Eds. (MIT Press, Cambridge, MA, 2000), pp. 713–737.
24. J. D. Cohen, T. S. Braver, J. W. Brown, *Curr. Opin. Neurobiol.* **12**, 223 (2002).
25. J. K. Seamans, C. R. Yang, *Prog. Neurobiol.* **74**, 1 (2004).
26. D. Servan-Schreiber, H. Printz, J. D. Cohen, *Science* **249**, 892 (1990).
27. M. J. Frank, B. Loughry, R. C. O'Reilly, *Cognit. Affect. Behav. Neurosci.* **1**, 137 (2001).
28. J. W. Mink, *Prog. Neurobiol.* **50**, 381 (1996).
29. R. C. O'Reilly, M. J. Frank, *Neural Comput.* **18**, 283 (2006).
30. T. E. Hazy, M. J. Frank, R. C. O'Reilly, *Neuroscience* **139**, 105 (2006).
31. N. P. Rougier, D. Noelle, T. S. Braver, J. D. Cohen, R. C. O'Reilly, *Proc. Natl. Acad. Sci. U.S.A.* **102**, 7338 (2005).
32. I. M. White, S. P. Wise, *Exp. Brain Res.* **126**, 315 (1999).
33. J. D. Wallis, K. C. Anderson, E. K. Miller, *Nature* **411**, 953 (2001).
34. K. Sakai, R. E. Passingham, *Nat. Neurosci.* **6**, 75 (2003).
35. M. Tomasello, *The Cultural Origins of Human Cognition* (Harvard Univ. Press, Cambridge, MA, 2001).
36. P. R. Huttenlocher, *Neuropsychologia* **28**, 517 (1990).
37. J. B. Morton, Y. Munakata, *Dev. Sci.* **5**, 435 (2002).
38. F. van der Velde, M. de Kamps, *Behav. Brain Sci.* **29**, 37 (2006).
39. J. R. Anderson *et al.*, *Psychol. Rev.* **111**, 1036 (2004).
40. J. R. Anderson, *How Can the Human Mind Occur in the Physical Universe?* (Oxford Univ. Press, New York, 2006).
41. Supported by Defense Advanced Research Projects Agency/Office of Naval Research grants N00014-05-1-0880 and N00014-03-1-0428 and NIH grants MH069597 and MH64445. I thank my long-time collaborators in developing these ideas: J. Cohen, T. Braver, D. Noelle, M. Frank, Y. Munakata, and the CCN Lab at the University of Colorado.

10.1126/science.1127242

Generation of Simian-Tropic HIV-1 by Restriction Factor Evasion

Theodora Hatziioannou,[1] Michael Princiotta,[2] Michael Piatak Jr.,[2] Fang Yuan,[2] Fengwen Zhang,[1] Jeffrey D. Lifson,[2] Paul D. Bieniasz[1]*

Although the evolution of intrinsic cellular defenses has likely prevented colonization of humans by many viruses (*1*), it also poses problems for AIDS research, insofar as the same intrinsic factors may inhibit HIV-1 replication in animal models. Indeed, HIV-1 does not replicate in most nonhuman primates, so research has relied on animal models of AIDS that are based on related simian viruses, which have inherent limitations. HIV-1 infection of rhesus macaque (rh) cells in vitro fails in part because a block is imposed by the cellular factor rhTRIM5α, which targets incoming HIV-1 capsids (CA) (*2*). A second block may be imposed by cellular APOBEC3 cytidine deaminase enzymes (*3*) because of a failure of HIV-1 Vif to bind rhAPOBEC3G and induce its degradation as it does in human cells (*4*). However, it is not known whether overcoming these blocks would allow HIV-1 replication in rhesus macaque cells.

In an attempt to generate a simian-tropic HIV-1, we incorporated sequences from a simian lentivirus (SIV_{MAC239}) into HIV-1 molecular clones. In these chimeric HIV-1–based genomes, "SCA" and "SVif" indicate that the CA and Vif sequences originated from SIV_{MAC239} (Fig. 1A). Replacement of HIV-1 CA, generating HIV(SCA) (*5*), attenuated replication, but after serial in vitro passage in human CEMx174 cells (19 times over 16 weeks) HIV(SCA) replicated robustly and could rapidly induce abundant cytopathic effects, unlike the starting virus. A fragment of this adapted HIV(SCA) genome (BssHII-ApaI, Fig. 1A) was reintroduced into an HIV-1 genomic clone, encoding green fluorescent protein (GFP) in place of the nonessential Nef protein, to generate an improved and easily monitored HIV(SCA) virus (*5*).

Subsequently, HIV-1 *vif* was replaced to generate viral clones, namely HIV, HIV(SCA), HIV(SVif), and HIV(SCA, SVif), encoding the four possible combinations of HIV-1 and SIV_{MAC239} CA and Vif proteins. In single-cycle infection assays (Fig. 1B), expression of rhAPOBEC3G during virus production reduced HIV-1 Vif–encoding virus infectivity by >30-fold but only marginally affected SIV_{MAC239} Vif–encoding counterparts. Conversely, rhTRIM5α expression in target cells inhibited HIV-1 CA–encoding virus infection by about 30-fold, but SIV_{MAC239} CA–encoding viruses were unaffected (Fig. 1B).

HIV, HIV(SCA), and HIV(SVif) replicated rapidly in CEMx174 cells, whereas HIV(SCA, SVif) replicated more slowly. Conversely, HIV and HIV(SVif) did not replicate in a rhesus macaque T cell line, 221, whereas HIV(SCA) replication was low and transient (fig. S1). Only HIV(SCA, SVif) initiated a slowly spreading infection in 221 cells, with cytopathic effects becoming evident at 16 to 20 days postinfection (fig. S1). HIV(SCA, SVif) replication became robust after adaptation (two passages in CEMx174 cells and three passages in 221 cells) and, thereafter, a BssHII-SalI fragment of this adapted HIV(SCA, SVif) genome was reintroduced into an HIV-1 genome encoding Nef, generating the simian tropic HIV-1 (stHIV-1) clone. Importantly, stHIV-1 differed from the parental HIV-1 equivalent in only minor ways, other than the CA and Vif substitutions. Specifically, stHIV-1 had few coding (amino acids Lys^{110}→Ile^{110}, Ala^{208}→Val^{208}, and Pro^{371}→Leu^{371}) and silent mutations in *gag* (nucleotides 291, 321, and 477) and in *pol* (nucleotides 1248, 2157, and 3411). No sequence changes in or around *vif* were evident.

The stHIV-1 clone replicated robustly in both human CEMx174 cells and macaque 221 cells (Fig. 1C). Moreover, stHIV-1 and SIV_{MAC239} replicated with similar rapid kinetics in peripheral blood lymphocytes (PBL) from all five macaque donors tested (Fig. 1C). In contrast, HIV-1 replication was either extremely low (two donors) or undetectable (three donors).

Overall, these findings suggest that avoidance of CA- and Vif-based restriction, by chance or adaptation, may be sufficient to allow cross-species transmission of primate lentiviruses. Additionally, the generation of a macaque cell-tropic virus whose genome is 88% HIV-1 derived should allow the development of more authentic animal models of human AIDS and facilitate the preclinical development of new therapies and vaccines.

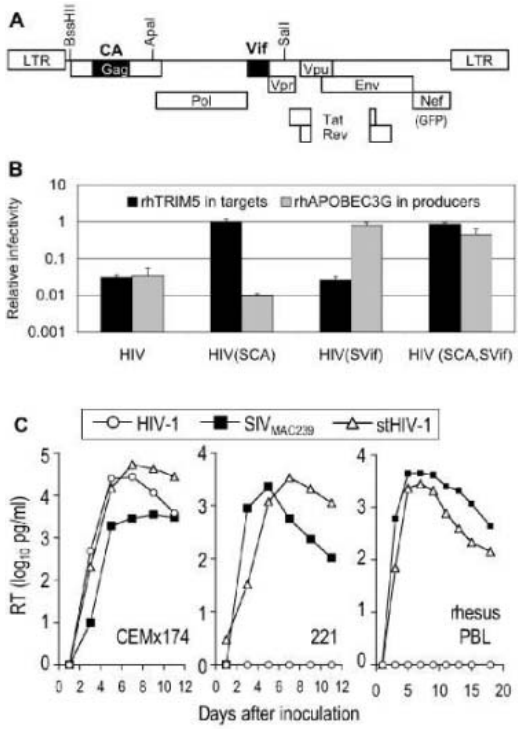


Fig. 1. (**A**) Schematic of the HIV(SCA, SVif) or stHIV-1 genome. Shaded regions are from SIV_{MAC239}. (**B**) Single-cycle infectivity of GFP-expressing HIV-1 and derivatives measured by using target CEMx174 cells expressing rhTRIM5α (black). Alternatively, viruses were generated in 293T cells in the presence of rhAPOBEC3G (gray). Values are plotted as a proportion of those obtained by using producer and target cells that did not express rhTRIM5α or rhAPOBEC3G. Error bars indicate standard deviations. (**C**) Reverse transcriptase (RT) accumulation in culture supernatants of human cells (CEMx174) or rhesus cells (221 and PBL) after challenge with HIV-1, SIV_{MAC239}, or stHIV-1.

References and Notes

1. P. D. Bieniasz, *Nat. Immunol.* **5**, 1109 (2004).
2. M. Stremlau *et al.*, *Nature* **427**, 848 (2004).
3. A. M. Sheehy, N. C. Gaddis, J. D. Choi, M. H. Malim, *Nature* **418**, 646 (2002).
4. R. Mariani *et al.*, *Cell* **114**, 21 (2003).
5. Materials and methods are available as supporting material on *Science* Online.

6. We thank V. KewalRamani, Z. Ambrose, D. Schneider, J. Smedley, and V. Coalter for advice and assistance and R. Desrosiers, J. Sodroski, and the NIH AIDS Reference Reagent Program for materials. Supported by the NIH, American Foundation for AIDS Research, and the Elizabeth Glaser Pediatric AIDS Foundation.

Supporting Online Material

www.sciencemag.org/cgi/content/full/314/5796/95/DC1
Materials and Methods
Fig. S1
References

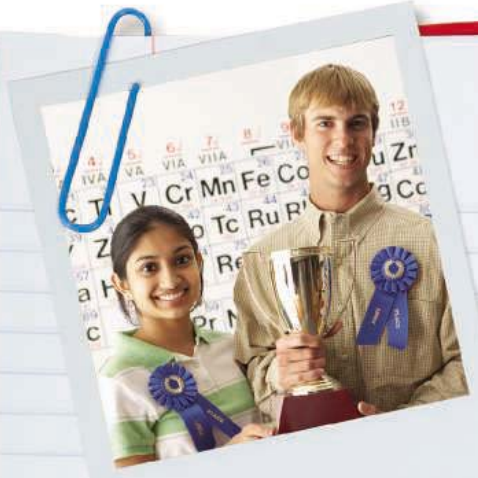
7 June 2006; accepted 2 August 2006
10.1126/science.1130994

[1]Aaron Diamond AIDS Research Center and Rockefeller University, 455 First Avenue, New York, NY 10016, USA. [2]AIDS Vaccine Program, SAIC Frederick, Incorporated, National Cancer Institute, Frederick, MD 21702, USA.

*To whom correspondence should be addressed. E-mail: pbienias@adarc.org

Call for Entries

Student Poster Competition



PUT YOURSELF IN THE PICTURE.

2007 AAAS Annual Meeting
15-19 February, San Francisco

"Science and Technology for Sustainable Well-Being"

Put yourself in the picture by presenting a poster at the AAAS Student Poster Session. It provides a great opportunity to present your research and offers an excellent venue for extended informal discussion with meeting attendees.

Accepted posters will be listed in the 2007 AAAS Meeting Poster Book. Winners will be published in *Science*. Free registration opportunities are available as are travel assistance grants, please see the Web site for futher details.

Deadline for submission: **Monday, 13 November 2006.**
See the Web site for full details: **www.aaasmeeting.org/student.**

"To raise new questions, new possibilities, to regard old problems from a new angle, requires creative imagination and marks real advance in science." Albert Einstein

www.aaasmeeting.org/student

AAAS
ADVANCING SCIENCE, SERVING SOCIETY

Tests of General Relativity from Timing the Double Pulsar

M. Kramer,[1]* I. H. Stairs,[2] R. N. Manchester,[3] M. A. McLaughlin,[1,4] A. G. Lyne,[1] R. D. Ferdman,[2] M. Burgay,[5] D. R. Lorimer,[1,4] A. Possenti,[5] N. D'Amico,[5,6] J. M. Sarkissian,[3] G. B. Hobbs,[3] J. E. Reynolds,[3] P. C. C. Freire,[7] F. Camilo[8]

The double pulsar system PSR J0737-3039A/B is unique in that both neutron stars are detectable as radio pulsars. They are also known to have much higher mean orbital velocities and accelerations than those of other binary pulsars. The system is therefore a good candidate for testing Einstein's theory of general relativity and alternative theories of gravity in the strong-field regime. We report on precision timing observations taken over the 2.5 years since its discovery and present four independent strong-field tests of general relativity. These tests use the theory-independent mass ratio of the two stars. By measuring relativistic corrections to the Keplerian description of the orbital motion, we find that the "post-Keplerian" parameter *s* agrees with the value predicted by general relativity within an uncertainty of 0.05%, the most precise test yet obtained. We also show that the transverse velocity of the system's center of mass is extremely small. Combined with the system's location near the Sun, this result suggests that future tests of gravitational theories with the double pulsar will supersede the best current solar system tests. It also implies that the second-born pulsar may not have formed through the core collapse of a helium star, as is usually assumed.

Einstein's general theory of relativity (GR) has so far passed all experimental tests with flying colors (*1*), with the most precise tests achieved in the weak-field gravity conditions of the solar system (*2*, *3*). However, it is conceivable that GR breaks down under extreme conditions such as strong gravitational fields where other theories of gravity may apply (*4*). Predictions of gravitational radiation and self-gravitational effects can only be tested using massive and compact astronomical objects such as neutron stars and black holes. Studies of the double–neutron star binary systems PSR B1913+16 and PSR B1534+12 have provided the best such tests so far, confirming GR at the 0.2% and 0.7% level, respectively (*5*–*7*). The recently discovered double pulsar system PSR J0737-3039A/B has much higher mean orbital velocities and accelerations than either PSR B1913+16 or PSR B1534+12 and is unique in that both neutron stars are detectable as radio pulsars (*8*, *9*).

[1]University of Manchester, Jodrell Bank Observatory, Macclesfield SK11 9DL, UK. [2]Department of Physics and Astronomy, University of British Columbia, 6224 Agricultural Road, Vancouver, British Columbia V6T 1Z1, Canada. [3]Australia Telescope National Facility, Commonwealth Scientific and Industrial Research Organisation (CSIRO), P.O. Box 76, Epping, New South Wales 1710, Australia. [4]Department of Physics, West Virginia University, Morgantown, WV 26505, USA. [5]INAF–Osservatorio Astronomica di Cagliari, Loc. Poggio dei Pini, Strada 54, 09012 Capoterra, Italy. [6]Dipartimento di Fisica, Università degli Studi di Cagliari, SP Monserrato-Sestu km 0.7, 09042 Monserrato (CA), Italy. [7]NAIC, Arecibo Observatory, HC03 Box 53995, PR 00612, USA. [8]Columbia Astrophysics Laboratory, Columbia University, 550 West 120th Street, New York, NY 10027, USA.

*To whom correspondence should be addressed. E-mail: mkramer@jb.man.ac.uk

PSR J0737-3039A/B consists of a pulsar with a period of 22 ms, PSR J0737-3039A (henceforth called A), in a 2.4-hour orbit with a younger pulsar with a period of 2.7 s, PSR J0737-3039B (B). Soon after the discovery of A (*8*), it was recognized that the orbit's orientation, measured as the longitude of periastron ω, was changing in time with a very large rate of $\dot{\omega} = d\omega/dt \sim 17^\circ\ \mathrm{year}^{-1}$, which exceeds by a factor of 4 the corresponding value for the Hulse-Taylor binary PSR B1913+16 (*5*). This immediately suggested that the system consists of two neutron stars, a conclusion confirmed by the discovery of pulsations from B (*9*). The pulsed radio emission from B has a strong orbital modulation, both in intensity and in pulse shape. It appears as a strong radio source only for two intervals, each of about 10-min duration, whereas its pulsed emission is rather weak or even undetectable for most of the remainder of the orbit (*9*, *10*).

In double–neutron star systems, especially those having short orbital periods, observed pulse arrival times are modified by relativistic effects that can be modeled in a theory-independent way by means of the so-called "post-Keplerian" (PK) parameters (*11*). These PK parameters are phenomenological corrections and additions to the simple Keplerian description of the binary motion, describing for instance a temporal change in period or orientation of the orbit, or an additional "Shapiro delay" that occurs as a result of the curvature of space-time when pulses pass near the massive companion. The PK parameters take different forms in different theories of gravity, and so their measurement can be used to test these theories (*1*, *12*). For point masses with negligible spin contributions, GR predicts values for the PK parameters that depend only on the two a priori unknown neutron star masses and the precisely measurable Keplerian parameters. Therefore, measurement of three (or more) PK parameters provides one (or more) test of the predictive power of GR. For the double pulsar we can also measure the mass ratio of the two stars, $R \equiv m_A/m_B = x_B/x_A$, where x_A and x_B are the projected semimajor axes of the orbits of A and B. The ability to measure this quantity provides an important constraint because in GR and other theories this simple relationship between the masses and semimajor axes is valid to at least first post-Newtonian (1PN) or $(v/c)^2$ order, where v is the orbital speed of the pulsars and *c* is the speed of light (*12*, *13*).

Observations. Timing observations of PSR J0737-3039A/B were undertaken using the 64-m Parkes radio telescope in New South Wales, Australia; the 76-m Lovell radio telescope at Jodrell Bank Observatory (JBO), UK; and the 100-m Green Bank Telescope (GBT) in West Virginia, USA, between April 2003 and January 2006.

At Parkes, observations were carried out in bands centered at 680 MHz, 1374 MHz, and 3030 MHz. Although timing observations were frequent after the discovery of the system, later observations at Parkes were typically conducted every 3 to 4 weeks, usually covering two full orbits per session. Observations at GBT were conducted at monthly intervals, with each session consisting of a 5- to 8-hour track (i.e., two to three orbits of the double pulsar). Typically, the observing frequencies were 820 and 1400 MHz for alternate sessions. Occasionally, we also performed observations at 340 MHz. In addition, we conducted concentrated campaigns of five 8-hour observing sessions, all at 820 MHz, in May and November 2005. Observations at JBO used the 76-m Lovell telescope. Most data were recorded at 1396 MHz; some observing sessions were carried out at the lower frequency of 610 MHz. The timing data obtained at JBO represent the most densely sampled data set, but because of the limited bandwidth, longer integration times per timing point were required. The Parkes data set is the longest one available and hence provides an excellent basis for investigation of secular timing terms.

The time-series data of all systems were folded according to the predicted topocentric pulse period. The adopted integration times were 30 s for pulsar A (180 s for JBO data) and 300 s for pulsar B. For A, these integration times reflected a compromise between the need to produce pulse profiles with adequate signal-to-noise ratio and to obtain sufficient sampling of the orbit to detect and resolve phenomena that depend on orbital phase, such as the Shapiro delay. The integration time for B corresponded to about 108 pulse periods and was a compromise between the need to form a stable pulse profile and to resolve the systematic changes seen as a function of orbital phase.

Timing measurements. For each of the final profiles, pulse times-of-arrival (TOAs) were computed by correlating the observed pulse profiles with synthetic noise-free templates (fig. S1) (*14*). A total of 131,416 pulse TOAs were measured for A; 507 TOAs were obtained for B. For A, the same template was used for all observations in a given frequency band, but different templates were used for widely separated bands. We note that our observations still provide no good evidence for secular evolution of A's profile (*15*) despite the predictions of geodetic precession. The best timing precision was obtained at 820 MHz with the Green Bank Astronomical Signal Processor (GASP) back end [see (*16*) for details of this and other observing systems] on GBT, with typical TOA measurement uncertainties for pulsar A of 18 μs for a 30-s integration.

For B, because of the orbital and secular dependence of its pulse profile (*10*), different templates were also used for different orbital phases and different epochs. A matrix of B templates was constructed, dividing the data set into 3-month intervals in epoch and 5-min intervals in orbital phase. The results for the 29 orbital phase bins were studied, and we noticed that although the profile changed quickly during the two prominent bright phases, the profile shape was simpler and more stable at orbital phases when the pulsar is weak. This apparent stability at some orbital phases cannot be attributed to a low signal-to-noise ratio, as secular variations in the pulse shape were still evident. Consequently, the orbital phase was divided into five groups of different lengths to which the same template (for a given 3-month interval) was applied as shown in fig. S2. In the final timing analysis, data from the two groups representing the bright phases (IV and V in fig. S2) were excluded to minimize the systematic errors caused by the orbital profile changes. Also, because of signal-to-noise and radio interference considerations, only data from Parkes and the GBT BCPM (Berkeley-Caltech Pulsar Machine) back end (*16*) were used in the B timing analysis.

All TOAs were transferred to Universal Coordinated Time (UTC) using the Global Positional System (GPS) to measure offsets of station clocks from national standards and Circular T of the Bureau International des Poids et Mesures (BIPM) to give offsets from UTC, and then to the nominally uniform BIPM Terrestrial Time (TT) time scale. These final TOAs were analyzed using the standard software package Tempo (*17*), fitting parameters according to the relativistic and theory-independent timing model of Damour and Deruelle (DD) (*11*, *18*). In addition to the DD model, we also applied the "DD-Shapiro" (DDS) model introduced by Kramer *et al.* (*19*). The DDS model is a modification of the DD model designed for highly inclined orbits. Rather than fitting for the Shapiro parameter s, the model uses the parameter $z_s \equiv -\ln(1 - s)$, which gives a more reliable determination of the uncertainties in z_s and hence in s. We quote the final result for the more commonly used parameter s and note that its value computed from z_s is in good agreement with the value obtained from a direct fit for s within the DD model. Derived pulsar and binary system parameters are listed in Table 1.

In the timing analysis for pulsar B, we used an unweighted fit to avoid biasing the fit toward bright orbital phases. Uncertainties in the timing parameters were estimated using Monte Carlo simulations of fake data sets for a range of TOA uncertainties, ranging from the minimum estimated TOA error to its maximum observed value of about 4 ms. For B, we also fitted for offsets between data sets derived from different templates in the fit because the observed profile changes prevent the establishment of a reliable phase relationship between the derived templates. This precludes a coherent fit across the whole orbit and hence limits the final timing precision for B. It cannot yet be excluded that different parts of B's magnetosphere are active and responsible for the observed emission at different orbital phases.

In the final fit, we adopted the astrometric parameters and the dispersion measure derived for A and held these fixed during the fit, because A's shorter period and more stable profile give much better timing precision than is achievable for B. Except for the semimajor axis—which is observable only as the projection onto the plane of the sky $x_B = (a_B/c)\sin i$, where a_B is

Table 1. Parameters for PSR J0737-3039A (A) and PSR J0737-3039B (B). The values were derived from pulse timing observations using the DD (*11*) and DDS (*19*) models of the timing analysis program Tempo and the Jet Propulsion Laboratory DE405 planetary ephemeris (*41*). Estimated uncertainties, given in parentheses after the values, refer to the least significant digit of the tabulated value and are twice the formal 1σ values given by Tempo. The positional parameters are in the DE405 reference frame, which is close to that of the International Celestial Reference System. Pulsar spin frequencies $\nu \equiv 1/P$ are in barycentric dynamical time (TDB) units at the timing epoch quoted in modified Julian days (MJD). The five Keplerian binary parameters (P_b, e, ω, T_0, and x) are derived for pulsar A. The first four of these (with an offset of 180° added to ω) and the position parameters were assumed when fitting for B's parameters. Five post-Keplerian parameters have now been measured. An independent fit of $\dot{\omega}$ for B yielded a value (shown in square brackets) that is consistent with the much more precise result for A. The value derived for A was adopted in the final analysis (*16*). The dispersion-based distance is based on a model for the interstellar electron density (*26*).

Timing parameter	PSR J0737-3039A	PSR J0737-3039B
Right ascension α	$07^h37^m51^s.24927(3)$	—
Declination δ	−30°39′40″.7195(5)	—
Proper motion in the RA direction (mas year^{-1})	−3.3(4)	—
Proper motion in declination (mas year^{-1})	2.6(5)	—
Parallax π (mas)	3(2)	—
Spin frequency ν (Hz)	44.054069392744(2)	0.36056035506(1)
Spin frequency derivative $\dot{\nu}$ (s^{-2})	$-3.4156(1) \times 10^{-15}$	$-0.116(1) \times 10^{-15}$
Timing epoch (MJD)	53,156.0	53,156.0
Dispersion measure DM (cm^{-3} pc)	48.920(5)	—
Orbital period P_b (day)	0.10225156248(5)	—
Eccentricity e	0.0877775(9)	—
Projected semimajor axis $x = (a/c)\sin i$ (s)	1.415032(1)	1.5161(16)
Longitude of periastron ω (°)	87.0331(8)	87.0331 + 180.0
Epoch of periastron T_0 (MJD)	53,155.9074280(2)	—
Advance of periastron $\dot{\omega}$ (°/year)	16.89947(68)	[16.96(5)]
Gravitational redshift parameter γ (ms)	0.3856(26)	—
Shapiro delay parameter s	0.99974(−39,+16)	—
Shapiro delay parameter r (μs)	6.21(33)	—
Orbital period derivative $\dot{P}_b$	$-1.252(17) \times 10^{-12}$	—
Timing data span (MJD)	52,760 to 53,736	52,760 to 53,736
Number of time offsets fitted	10	12
RMS timing residual σ (μs)	54	2169
Total proper motion (mas year^{-1})	4.2(4)	
Distance d(DM) (pc)	~500	
Distance d(π) (pc)	200 to 1,000	
Transverse velocity (d = 500 pc) (km s^{-1})	10(1)	
Orbital inclination angle (°)	88.69(−76,+50)	
Mass function ($M_\odot$)	0.29096571(87)	0.3579(11)
Mass ratio R	1.0714(11)	
Total system mass ($M_\odot$)	2.58708(16)	
Neutron star mass ($m_\odot$)	1.3381(7)	1.2489(7)

the semimajor axis of B's orbit and i is the orbital inclination angle—we also adopted A's Keplerian parameters (with 180° added to ω_A) and kept these fixed. We also adopted the PK parameter $\dot{\omega}$ (the rate of periastron advance) from the A fit because logically this must be identical for the two pulsars; this equality therefore does not implicitly make assumptions about the validity of any particular theory of gravity (see below). The same applies for the orbital decay parameter $\dot{P}_b$. In contrast, the PK parameters γ (the gravitational redshift and time dilation parameter) and s and r (the Shapiro-delay parameters) are asymmetric in the masses, and their values and interpretations differ for A and B. In practical terms, the relatively low timing precision for B does not require the inclusion of γ, s, r, or $\dot{P}_b$ in the timing model. We can, however, independently measure $\dot{\omega}_B$, obtaining a value of 16.96° ± 0.05° year^{-1}, consistent with the more accurately determined value for A.

Because the overall precision of our tests of GR is currently limited by our ability to measure x_B and hence the mass ratio $R \equiv m_A/m_B = x_B/x_A$ (see below), we adopted the following strategy to obtain the best possible accuracy for this parameter. We used the whole TOA data set for B in order to measure B's spin parameters P and $\dot{P}$, given in Table 1. These parameters were then kept fixed for a separate analysis of the concentrated 5-day GBT observing sessions at 820 MHz. On the time scale of the long-term profile evolution of B, each 5-day session represents a single-epoch experiment and hence requires only a single set of profile templates. The value of x_B obtained from a fit of this parameter only to the two 5-day sessions is presented in Table 1.

Because of the possible presence of unmodeled intrinsic pulsar timing noise and because not all TOA uncertainties are well understood, we adopt the common and conservative pulsar-timing practice of reporting twice the parameter uncertainties given by tempo as estimates of the 1σ uncertainties. Although we believe that our real measurement uncertainties are actually somewhat smaller than quoted, this practice facilitates comparison with previous tests of GR by pulsar observation. The timing model also includes timing offsets between the data sets for the different instruments represented by the entries in table S1. The final weighted root mean square post-fit residual is 54.2 μs. In addition to the spin and astrometric parameters, the Keplerian parameters of A's orbit, and five PK parameters, we also quote a tentative detection of a timing annual parallax that is consistent with the dispersion-derived distance. Further details are given in (*16*).

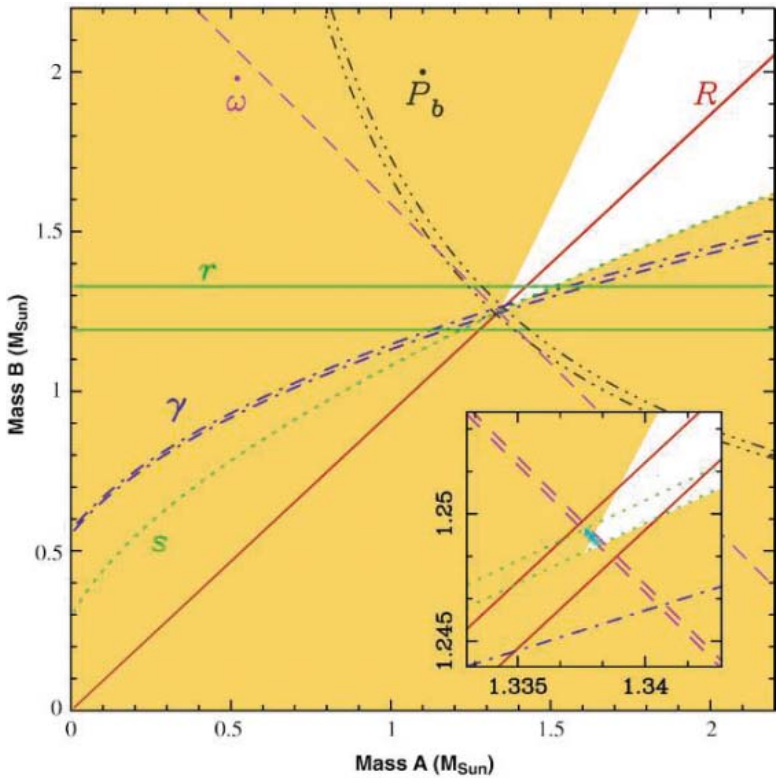


Fig. 1. Graphical summary of tests of GR parameters. Constraints on the masses of the two stars (A and B) in the PSR J0737-3039A/B binary system are shown; the inset is an expanded view of the region of principal interest. Shaded regions are forbidden by the individual mass functions of A and B because sin i must be ≤1. Other constraining parameters are shown as pairs of lines, where the separation of the lines indicates the measurement uncertainty. For the diagonal pair of lines labeled as R, representing the mass ratio derived from the measured semimajor axes of the A and B orbits, the measurement precision is so good that the line separation becomes apparent only in the inset. The other constraints shown are based on the measured PK parameters interpreted within the framework of general relativity. The PK parameter $\dot{\omega}$ describes the relativistic precession of the orbit, γ combines gravitational redshift and time dilation, and $\dot{P}_b$ represents the measured decrease in orbital period due to the emission of gravitational waves. The two PK parameters s and r reflect the observed Shapiro delay, describing a delay that is added to the pulse arrival times when propagating through the curved space-time near the companion. The intersection of all line pairs is consistent with a single point that corresponds to the masses of A and B. The current uncertainties in the observed parameters determine the size of this intersection area, which is marked in blue and reflects the achieved precision of this test of GR and the mass determination for A and B.

Tests of general relativity. Previous observations of PSR J0737-3039A/B (*8*, *9*) resulted in the measurement of R and four PK parameters: $\dot{\omega}$, γ, r, and s. Relative to these earlier results, the measurement precision for these parameters from PSR J0737-3039A/B has increased by up to two orders of magnitude. Also, we have now measured the orbital decay $\dot{P}_b$. Its value, measured at the 1.4% level after only 2.5 years of timing, corresponds to a shrinkage of the pulsars' separation at a rate of 7 mm per day. Therefore, we have measured five PK parameters for the system in total. Together with the mass ratio R, we have six different relationships that connect the two unknown masses for A and B with the observations. Solving for the two masses using R and one PK parameter, we can then use each further PK parameter to compare its observed value with that predicted by GR for the given two masses, providing four independent tests of GR. Equivalently, one can display these tests elegantly in a "mass-mass" diagram (Fig. 1). Measurement of the PK parameters gives curves on this diagram that are, in general, different for different theories of gravity but should intersect in a single point (i.e., at a pair of mass values) if the theory is valid (*12*).

As shown in Fig. 1, we find that all measured constraints are consistent with GR. The most precisely measured PK parameter currently available is the precession of the longitude of periastron, $\dot{\omega}$. We can combine this with the theory-independent mass ratio R to derive the masses given by the intersection region of their curves: $m_A = 1.3381 \pm 0.0007\ M_\odot$ and $m_B = 1.2489 \pm 0.0007\ M_\odot$, where $M_\odot$ is the mass of the Sun (*20*). Table 2 lists the resulting four independent tests that are currently available. All of them rely on comparison of our measured values of s, r, γ, and $\dot{P}_b$ with predicted values based on the masses defined by the intersection of the allowed regions for $\dot{\omega}$ and R in the m_A-m_B plane. The calculation of the predicted values is somewhat complicated by the fact that the orbit is nearly edge-on to the line of sight, so that the formal intersection region

actually includes parts of the plane disallowed by the Keplerian mass functions of both pulsars (see Fig. 1). To derive legitimate predictions for the various parameters, we used the following Monte Carlo method. A pair of trial values for $\dot{\omega}$ and x_B (and hence R and the B mass function) is selected from Gaussian distributions based on the measured central values and uncertainties. (The uncertainty on x_A is very small and is neglected in this procedure.) This pair of trial values is used to derive trial masses m_A and m_B, using the GR equation $\dot{\omega} = 3(P_b/2\pi)^{-5/3}(T_\odot M)^{2/3}(1 - e^2)^{-1}$, where e is the orbital eccentricity and $M = m_A + m_B$ and $T_\odot \equiv GM_\odot/c^3 = 4.925490947$ μs, and the mass-ratio equation $m_A/m_B = x_B/x_A$. If this trial mass pair falls in either of the two disallowed regions (based on the trial mass function for B), it is discarded. This procedure allows for the substantial uncertainty in the B mass function. Allowed mass pairs are then used to compute the other PK parameters, assuming GR. This procedure is repeated until large numbers of successful trials have accumulated. Histograms of the PK predictions are used to compute the expectation value and 68% confidence ranges for each of the parameters. These are the values given in Table 2.

The Shapiro delay shape illustrated in Fig. 2 gives the most precise test, with $s_{observed}/s_{predicted} = 0.99987 \pm 0.00050$ (*21*). This is by far the best available test of GR in the strong-field limit, having a higher precision than the test based on the observed orbit decay in the PSR B1913+16 system with a 30-year data span (*22*). As for the PSR B1534+12 system (*6*), the PSR J0737-3039A/B Shapiro-delay test is complementary to that of B1913+16 because it is not based on predictions relating to emission of gravitational radiation from the system (*23*). Most important, the four tests of GR presented here are qualitatively different from all previous tests because they include one constraint (R) that is independent of the assumed theory of gravity at the 1PN order. As a result, for any theory of gravity, the intersection point is expected to lie on the mass ratio line in Fig. 1. GR also passes this additional constraint.

In estimating the final uncertainty of x_B and hence of R, we have considered that geodetic precession will lead to changes to the system geometry and hence changes to the aberration of the rotating pulsar beam. The effects of aberration on pulsar timing are usually not separately measurable but are absorbed into a redefinition of the Keplerian parameters. As a result, the observed projected sizes of the semimajor axes, $x^{obs}_{A,B}$, differ from the intrinsic sizes, $x^{int}_{A,B}$, by a factor $(1 + \varepsilon^A_{A,B})$. The quantity ε_A depends for each pulsar A and B on the orbital period, the spin frequency, the orientation of the pulsar spin, and the system geometry (*12*). Although aberration should eventually become detectable in the timing, allowing the determination of a further PK parameter, at present it leads to an undetermined deviation of x^{obs} from x^{int}, where the latter is the relevant quantity for the mass ratio. The parameter $\varepsilon^A_{A,B}$ scales with pulse period and is therefore expected to be two orders of magnitude smaller for A than for B. However, because of the high precision of the A timing parameters, the derived value x^{obs}_A may already be significantly affected by aberration. This has (as yet) no consequences for the mass ratio $R = x^{obs}_B/x^{obs}_A$, as the uncertainty in R is dominated by the much less precise x^{obs}_B. We can explore the likely aberration corrections to x^{obs}_B for various possible geometries. Using a range of values given by studies of the double pulsar's emission properties (*24*), we estimate $\varepsilon^A_A \sim 10^{-6}$ and $\varepsilon^A_B \sim 10^{-4}$. The contribution of aberration therefore is at least one order of magnitude smaller than our current timing precision. In the future this effect may become important, possibly limiting the usefulness of R for tests of GR. If the geometry cannot be independently determined, we could use the observed deviations of R from the value expected within GR to determine ε^A_B and hence the geometry of B.

Space motion and inclination of the orbit. Because the measured uncertainty in $\dot{P}_b$ decreases approximately as $T^{-2.5}$, where T is the data span, we expect to improve our test of the radiative aspect of the system to the 0.1% level or better in about 5 years' time. For the PSR B1913+16 and PSR B1534+12 systems, the precision of the GR test based on the orbit-

Table 2. Four independent tests of GR provided by the double pulsar. Observed PK parameters were obtained by fitting a DDS timing model to the data. Values expected from GR take into account the masses determined from the intersection point of the mass ratio R and the periastron advance $\dot{\omega}$. Uncertainties refer to the last significant digits and were determined using Monte Carlo methods.

PK parameter	Observed value	Expected value from GR	Ratio of observed to expected value
$\dot{P}_b$	1.252(17)	1.24787(13)	1.003(14)
γ (ms)	0.3856(26)	0.38418(22)	1.0036(68)
s	0.99974(−39,+16)	0.99987(−48,+13)	0.99987(50)
r (μs)	6.21(33)	6.153(26)	1.009(55)

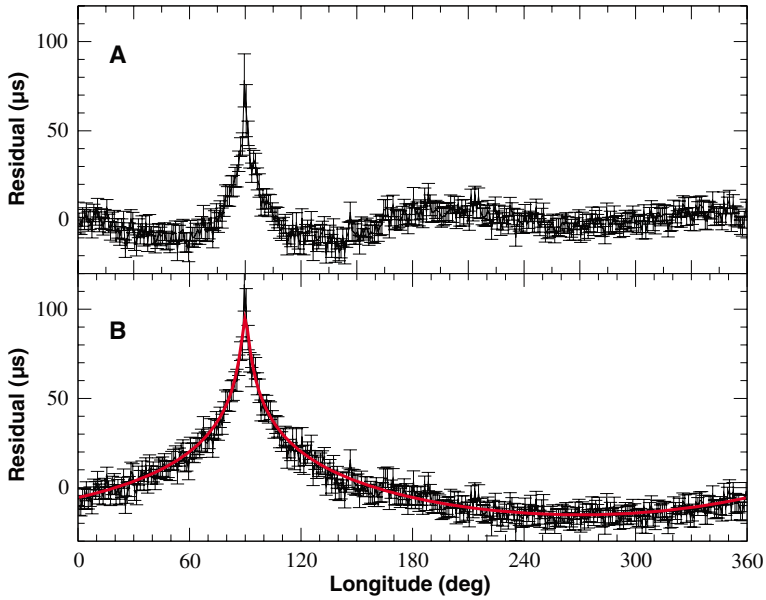


Fig. 2. Measurement of a Shapiro delay demonstrating the curvature of space-time. Timing residuals (differences between observed and predicted pulse arrival times) are plotted as a function of orbital longitude and illustrate the Shapiro delay for PSR J0737-3039A. (**A**) Observed timing residuals after a fit of all model parameters given in Table 1 except the Shapiro-delay terms r and s, which were set to zero and are not included in the fit. Although a portion of the delay is absorbed in an adjustment of the Keplerian parameters, a strong peak at 90° orbital longitude remains clearly visible. This is the orbital phase of A's superior conjunction (i.e., when it is positioned behind B as viewed from Earth), so that its pulses experience a delay when moving through the curved space-time near B. The clear detection of structure in the residuals over the whole orbit confirms the detection of the Shapiro delay, which is isolated in (**B**) by holding all parameters to their best-fit values given in Table 1, except the Shapiro delay terms (which were set to zero). The red line shows the predicted delay at the center of the data span. In both cases, residuals were averaged in 1° bins of longitude.

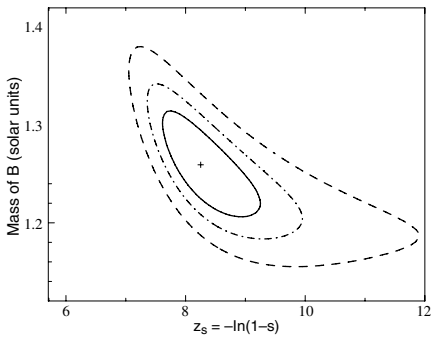


Fig. 3. Contour plots of the χ^2 distribution in the plane of the Shapiro-delay parameter $z_s \equiv -\ln(1 - s)$ and the mass of the B pulsar, m_B. The contours correspond to 68%, 95%, and 99% confidence limits.

decay rate is severely limited by the uncertainty in the differential acceleration of the Sun and the binary system in the galactic gravitational potential as well as by the uncertainty in pulsar distance (*6*, *25*). For PSR J0737-3039A/B, both of these corrections are very much smaller than for these other systems. On the basis of the measured dispersion measure and a model for the galactic electron distribution (*26*), PSR J0737-3039A/B is estimated to be about 500 pc from Earth. From the timing data we have measured a marginally significant value for the annual parallax, 3 ± 2 mas, corresponding to a distance of 200 to 1000 pc (Table 1), which is consistent with the dispersion-based distance that was also used for studies of detection rates in gravitational wave detectors (*8*). The observed proper motion of the system (Table 1) and differential acceleration in the galactic potential (*27*) then imply a kinematic correction to $\dot{P}_b$ at the 0.02% level or less. Independent distance estimates also can be expected from measurements of the annual parallax by very-long-baseline interferometry observations, allowing a secure compensation for this already small effect. A measurement of $\dot{P}_b$ at the 0.02% level or better will provide stringent tests for alternative theories of gravity. For example, limits on some scalar-tensor theories will surpass the best current solar system tests (*28*).

In GR, the parameter s can be identified with $\sin i$, where i is the inclination angle of the orbit. The value of s given in Table 1 corresponds to $i = 88°.69^{+0°.50}_{-0°.76}$. On the basis of scintillation observations of both pulsars over the short time interval when A is close to superior conjunction, Coles *et al*. (*29*) derived a value for $|i - 90°|$ of 0°.29 ± 0°.14. This is consistent with our measurement only at the 3σ level. As mentioned above, we used the DDS model to solve for the Shapiro delay. The resulting χ^2 contours in the z_s-m_B plane are shown in Fig. 3. The value and uncertainty range for s quoted in Table 1 correspond to the peak and range of the 68% contour. Because of the nonlinear relationship between z_s and s, the uncertainty distribution in s (and hence in i) corresponding to these contours is very asymmetric with a very steep edge on the 90° side. Only close to the 99% confidence limit is the timing result consistent with the scintillation-derived value. We note that the scintillation measurement is based on the correlation of the scintillation fluctuations of A and B over the short interval when A is close to superior conjunction (i.e., behind B). In contrast, the measurement of i from timing measurements depends on the detection of significant structure in the post-fit residuals after a portion of the Shapiro delay is absorbed in the fit for x_A (*30*). As shown in Fig. 2, the Shapiro delay has a signature that is spread over the whole orbit and hence can be cleanly isolated. We also examined the effects on the Shapiro delay of using only low- or high-frequency data, and we found values of s consistent within the errors in each case. The scintillation result is based on the plasma properties of the interstellar medium and may also be affected by possible refraction effects in B's magnetosphere. We believe that the timing result is much less susceptible to systematic errors and is therefore more secure.

Scintillation observations have also been used to deduce the system transverse velocity. Ransom *et al*. (*31*) derived a value of 141 ± 8.5 km s^{-1}, whereas Coles *et al*. (*29*) obtained 66 ± 15 km s^{-1} after considering the effect of anisotropy in the scattering screen. Both of these values are in stark contrast to the value of 10 ± 1 km s^{-1} (relative to the solar system barycenter) obtained from pulsar timing (Table 1). We note that the scintillation-based velocity depends on a number of assumptions about the properties of the effective scattering screen. In contrast, the proper motion measurement has a clear and unambiguous timing signature, although the transverse velocity itself scales with the pulsar distance. Even allowing that unmodeled effects of Earth motion could affect the published scintillation velocities by about 30 km s^{-1}, the dispersion-based distance would need to be underestimated by a factor of 3 to 4 to make the velocities consistent. We believe this is very unlikely, particularly as the tentative detection of a parallax gives us some confidence in the dispersion-based distance estimate. Hence, we believe that our timing results for both inclination angle and transverse velocity are less susceptible to systematic errors and are therefore more secure than those based on scintillation.

We note that because the inclination angle is significantly different from 90°, gravitational lensing effects (*32*) can be neglected. The implied low space velocity, the comparatively low derived mass for B, and the low orbit eccentricity are all consistent with the idea that the B pulsar may have formed by a mechanism different from the usually assumed core collapse of a helium star (*33*, *34*). A discussion of its progenitor is presented in (*35*). We also note that, as expected for a double–neutron star system, there is no evidence for variation in dispersion measure as a function of orbital phase.

Future tests. In contrast to all previous tests of GR, we are now reaching the point with PSR J0737-3039A where expressions of PK parameters to only 1PN order may no longer be sufficient for a comparison of theoretical predictions with observations. In particular, we have measured $\dot{\omega}$ so precisely (i.e., to a relative precision approaching 10^{-5}) that we expect corrections at the 2PN level (*13*) to be observationally significant within a few years. These corrections include contributions expected from spin-orbit coupling (*36*, *37*). A future determination of the system geometry and the measurement of two other PK parameters at a level of precision similar to that for $\dot{\omega}$ would allow us to measure the moment of inertia of a neutron star for the first time (*13*, *38*). Although this measurement is potentially very difficult, a determination of A's moment of inertia to a precision of only 30% would allow us to distinguish between a large number of proposed equations of state for dense matter (*39*, *40*). The double pulsar would then not only provide the best tests of theories of gravity in the strong-field regime, as presented here, but would also give insight into the nature of superdense matter.

References and Notes

1. C. Will, *Living Rev. Relativity* **4**, 4 (2001).
2. B. Bertotti, L. Iess, P. Tortora, *Nature* **425**, 374 (2003).
3. J. G. Williams, S. G. Turyshev, D. H. Boggs, *Phys. Rev. Lett.* **93**, 261101 (2004).
4. T. Damour, G. Esposito-Farèse, *Phys. Rev. D* **58**, 1 (1998).
5. J. H. Taylor, J. M. Weisberg, *Astrophys. J.* **345**, 434 (1989).
6. I. H. Stairs, S. E. Thorsett, J. H. Taylor, A. Wolszczan, *Astrophys. J.* **581**, 501 (2002).
7. Stairs *et al*. (*6*) found agreement of their measured values for PSR B1534+12 with GR at the 0.05% level, but the measurement uncertainty on the most precisely measured parameter in the test, s, was only 0.7%.
8. M. Burgay *et al.*, *Nature* **426**, 531 (2003).
9. A. G. Lyne *et al.*, *Science* **303**, 1153 (2004); published online 8 January 2004 (10.1126/science.1094645).
10. M. Burgay *et al.*, *Astrophys. J.* **624**, L113 (2005).
11. T. Damour, N. Deruelle, *Ann. Inst. H. Poincare Phys. Theor.* **44**, 263 (1986).
12. T. Damour, J. H. Taylor, *Phys. Rev. D* **45**, 1840 (1992).
13. T. Damour, G. Schäfer, *Nuovo Cim.* **101**, 127 (1988).
14. M. Kramer *et al.*, *Astrophys. J.* **526**, 957 (1999).
15. R. N. Manchester *et al.*, *Astrophys. J.* **621**, L49 (2005).
16. See supporting material on *Science* Online.
17. Tempo (www.atnf.csiro.au/research/pulsar/tempo).
18. T. Damour, N. Deruelle, *Ann. Inst. H. Poincare Phys. Theor.* **43**, 107 (1985).
19. M. Kramer *et al.*, *Ann. Phys.* **15**, 34 (2006).
20. The true masses will deviate from these values by an unknown, but essentially constant, Doppler factor, probably of order 10^{-3} or less (*12*). Moreover, what is measured is a product containing Newton's gravitational constant G. The relative uncertainty of G of 1.5×10^{-4} limits our knowledge of any astronomical mass in kilograms, but because the product $T_\odot = GM_\odot/c^3 = 4.925490947$ μs is known to very high precision, masses can be measured precisely in solar units.
21. Note that s has the same relative uncertainty as our determination of the masses.

22. J. M. Weisberg, J. H. Taylor, in *Binary Radio Pulsars*, F. Rasio, I. H. Stairs, Eds. (Astronomical Society of the Pacific, San Francisco, 2005), pp. 25–31.
23. J. H. Taylor, A. Wolszczan, T. Damour, J. M. Weisberg, *Nature* **355**, 132 (1992).
24. M. Lyutikov, *Mon. Not. R. Astron. Soc.* **362**, 1078 (2005).
25. T. Damour, J. H. Taylor, *Astrophys. J.* **366**, 501 (1991).
26. J. M. Cordes, T. J. W. Lazio, http://arxiv.org/abs/astro-ph/0207156 (2002).
27. K. Kuijken, G. Gilmore, *Mon. Not. R. Astron. Soc.* **239**, 571 (1989).
28. T. Damour, G. Esposito-Farèse, in preparation.
29. W. A. Coles, M. A. McLaughlin, B. J. Rickett, A. G. Lyne, N. D. R. Bhat, *Astrophys. J.* **623**, 392 (2005).
30. C. Lange *et al.*, *Mon. Not. R. Astron. Soc.* **326**, 274 (2001).
31. S. M. Ransom *et al.*, *Astrophys. J.* **609**, L71 (2004).
32. R. R. Rafikov, D. Lai, *Phys. Rev. D* **73**, 063003 (2006).
33. E. Pfahl, S. Rappaport, P. Podsiadlowski, H. Spruit, *Astrophys. J.* **574**, 364 (2002).
34. T. Piran, N. J. Shaviv, *Phys. Rev. Lett.* **94**, 051102 (2005).
35. I. H. Stairs, S. E. Thorsett, R. J. Dewey, M. Kramer, C. McPhee, *Mon. Not. R. Astron. Soc.*, in press (2006).
36. T. Damour, R. Ruffini, *C. R. Acad. Sci. Paris Ser. I Math.* **279**, 971 (1974).
37. B. M. Barker, R. F. O'Connell, *Astrophys. J.* **199**, L25 (1975).
38. N. Wex, *Class. Quantum Grav.* **12**, 983 (1995).
39. I. A. Morrison, T. W. Baumgarte, S. L. Shapiro, V. R. Pandharipande, *Astrophys. J.* **617**, L135 (2004).
40. J. M. Lattimer, B. F. Schutz, *Astrophys. J.* **629**, 979 (2005).
41. E. M. Standish, *Astron. Astrophys.* **336**, 381 (1998).
42. We thank T. Damour and N. Wex for useful discussions. The Parkes radio telescope is part of the Australia Telescope, which is funded by the Commonwealth of Australia for operation as a National Facility managed by CSIRO. The National Radio Astronomy Observatory (NRAO) is a facility of the NSF operated under cooperative agreement by Associated Universities Inc. GASP is funded by a Natural Sciences and Engineering Research Council of Canada (NSERC) RTI-1 grant (I.H.S.) and by NSF grants to D. Backer and D. Nice. We thank P. Demorest, R. Ramachandran, and J. van Leeuwen for their contributions to GASP hardware and software development. Supported by an NSERC University Faculty Award (I.H.S.), by the Italian Ministry of University and Research under the national program Cofin 2003 (M.B., A.P., and N.D'A.), and by NSF, NASA, and NRAO (F.C.). Pulsar research at the University of British Columbia is supported by an NSERC Discovery Grant.

Supporting Online Material

www.sciencemag.org/cgi/content/full/1132305/DC1
SOM Text
Figs. S1 to S4
Tables S1 and S2

10 July 2006; accepted 7 September 2006
Published online 14 September 2006;
10.1126/science.1132305
Include this information when citing this paper.

Out of the Tropics: Evolutionary Dynamics of the Latitudinal Diversity Gradient

David Jablonski,[1]* Kaustuv Roy,[2] James W. Valentine[3]

The evolutionary dynamics underlying the latitudinal gradient in biodiversity have been controversial for over a century. Using a spatially explicit approach that incorporates not only origination and extinction but immigration, a global analysis of genera and subgenera of marine bivalves over the past 11 million years supports an "out of the tropics" model, in which taxa preferentially originate in the tropics and expand toward the poles without losing their tropical presence. The tropics are thus both a cradle and a museum of biodversity, contrary to the conceptual dichotomy dominant since 1974; a tropical diversity crisis would thus have profound evolutionary effects at all latitudes.

The most striking large-scale pattern in biological diversity is the dramatic increase in the number of species and higher taxa from the poles to the tropics. This taxonomic trend, commonly called the latitudinal diversity gradient (LDG), has been documented in the multicellular biotas of forests, grasslands, wetlands, continental shelves, the open ocean, and even the deep sea; it characterizes plants, fungi, marine and freshwater invertebrates, and all of the vertebrate classes (*1*). The history of the LDG extends back through the Mesozoic into the Paleozoic (*2*–*7*), although the slope of the gradient has varied over time and the trend might even have disappeared for a time if any of the mass extinctions were disproportionately severe in the tropics (*8*).

Although the existence of the LDG has been known for more than a century (*9*, *10*) and has been quantified hundreds of times (*1*), it remains the "major, unexplained pattern of natural history" [Ricklefs in (*11*)], with "an astonishing lack of consensus about the mechanisms leading to this variation in diversity" (*1*). Recent work has focused primarily on ecological explanations for the LDG (*9*, *12*–*15*), and although these analyses have found interesting correlations between diversity and environmental variables, they reveal little about the evolutionary dynamics of the species and lineages that established and maintain the LDG (*16*, *17*). Because virtually all possible combinations of the key evolutionary parameters have been proposed to shape the LDG (table S1), progress in this area depends on empirical data that can falsify alternatives. Here we (i) outline a framework for evaluating the spatial and temporal dynamics that underlie the present-day LDG, (ii) synthesize previous work from this perspective, and (iii) present paleontological analyses that falsify the classic portrayal of the tropics as either a cradle or a museum of biodiversity (*18*).

Cradles and Museums

From an evolutionary perspective, large-scale spatial patterns of biodiversity depend on three variables: origination rates (O), extinction rates (E), and changes in geographic distributions (expressed here as I, for immigration into a latitudinal bin) of taxa. For a simple two-box model, with the tropics and extratropics denoted as subscripts, diversity in the tropics (D_T) is determined by $O_T - E_T + I_T$, and diversity in the extratropics (D_E) by $O_E - E_E + I_E$ (Fig. 1). With this notation, it can easily be seen that a latitudinal gradient in richness, with $D_T > D_E$, can result from many different combinations of these variables. Theoretically, the extinction terms could represent either true global extinction of taxa, local extinction for a particular spatial bin, or a combination of the two. Estimating local extinction rates using paleontological data is generally difficult owing to incomplete spatial sampling, and even more difficult using phylogenetic information. In addition, our empirical results suggest that the effect of local extinction is much smaller than that of range expansion, at least for marine bivalves. Thus, as in most previous studies (table S1), our discussion of the role of extinction in shaping the LDG focuses primarily on global processes.

The simplest evolutionary models for the LDG assume that taxa are static in their geographic distributions ($I_T = I_E = 0$) and treat the greater number of species and higher taxa in the tropics as the result of either a higher rate of origination of species and lineages ($O_T > O_E$) or lower extinction rates as compared to extratropical regions ($E_T < E_E$). For example, Wallace (*19*) attributed high tropical diversity to a more stable climatic history, which allowed more time to accumulate taxa ($E_T < E_E$), and this view has found proponents ever since (*20*) (table S1). Others have argued that extinction rates are high in the tropics but are outstripped by even higher origination rates ($E_T > E_E$, $O_T \gg O_E$) (*21*). The importance of origination and extinction in generating the LDG was highlighted in Stebbins' (*18*) famous metaphor of the tropics as a cradle or a museum, and this memorable dichotomy has been the dominant paradigm ever since.

[1]Department of Geophysical Sciences, University of Chicago, 5734 South Ellis Avenue, Chicago, IL 60637, USA. [2]Section of Ecology, Behavior and Evolution, University of California, San Diego, 9500 Gilman Drive, La Jolla, CA 92093–0116, USA. [3]Department of Integrative Biology, University of California, Berkeley, Berkeley, CA 94720, USA.

*To whom correspondence should be addressed. E-mail: djablons@uchicago.edu

Distinguishing evolutionary cradles from museums requires separate estimates of origination and extinction rates. Such estimates are currently unavailable even for most large groups with a good fossil record and may not be feasible for groups lacking a fossil record without assuming stochastically constant extinction rates (*22*), an assumption often violated over the past 15 million years of Cenozoic history (*23*, *24*). Consequently, attempts to quantify the evolutionary underpinnings of the LDG have focused mainly on latitudinal differences in net diversification rates of living taxa [the composite value ($O - E$)], a parameter more readily estimated from phylogenies of extant organisms (table S1) (*25*, *26*). Such differences in net diversification rates are valuable for investigating many questions (*26*), but their application to the cradle/museum problem is again limited by the many combinations of O and E that can produce a given net value. Realistically, areas with high net diversification rates are more likely to be evolutionary cradles, but those where such rates are low could have experienced either high or low extinction rates.

Rate Differences and Range Shifts

The cradle/museum dichotomy, and the more general hypothesis that attributes high tropical diversity to higher net diversification rates, implicitly assume that the LDG derives largely from differences in in situ origination and extinction (*16*, *25*, *26*). However, this simplifying assumption is contradicted by biogeographic data showing that (i) many taxa shift their geographic range limits substantially in response to climatic changes [they have moved across latitudes to track changing climates (*27*, *28*)], and (ii) many taxa have geographic distributions that encompass both tropical and extratropical regions [assuming origination in a single climate zone, they have expanded across latitudes in the face of climate differences (*9*, *29*)]. Thus, the dynamics underlying the LDG must involve not only latitudinal differences in origination and/or extinction rates but also extensive changes in spatial distributions of taxa over time.

Although most analyses of the LDG based on present-day biogeography have ignored the role of past distributional changes, the notion that shifts in latitudinal distributions of taxa play an important role in shaping the LDG is not new (*20*, *30*–*33*). Scenarios in which taxa preferentially originate in tropical regions and spread out from there ($I_T < I_E$) or the reverse ($I_T > I_E$) have both been advocated (*33*), but attempts to separate the contributions of O, E, and I to the shape of the LDG have been undermined by a lack of basic information on the time and place of origin for the vast majority of living taxa. Instead, taxa occurring in both tropical and extratropical regions are generally handled either by (i) including each taxon in rate calculations for all latitudinal bins within its geographic range (*34*) or (ii) including each taxon only in the bin corresponding to the center of its latitudinal range (*25*, *26*). Neither approach can separate the effects of past distributional shifts from those due to changes in diversification rates with latitude, however. Protocol (i) is analytically problematic (owing to the autocorrelation imposed by counting each taxon in multiple bins) and allows a widespread taxon to influence the age distributions of more latitudinal bins than a restricted taxon does, even though each should contribute only to its latitude of origin. In contrast, protocol (ii) makes the unrealistic assumption that taxa originate near the midpoint of their present-day geographic ranges. The asymmetry of range expansion from the true place of origin is likely to increase with the geographic range of a taxon (*26*), and even narrow-ranging taxa may abandon ancestral distributions in response to large climatic changes such as occurred during the Pleistocene (*27*). Some progress has been made recently in estimating origination, extinction, and immigration rates from the shapes of taxon age distributions, but such models also make a number of important simplifying assumptions about the underlying dynamics (*35*). Thus, direct tests of the role of large-scale range expansion in shaping the LDG are needed, and the fossil record remains the best source of data for such tests.

Out of the Tropics: A Dynamic Model

One potential reason why published studies have failed to produce a consensus on whether the tropics are a biological cradle or museum (table S1) is that this dichotomy is misleading. The tropics could be a cradle, a museum, or both; theoretically, so could the polar regions; and taxa could predominantly remain in place or either expand or contract their distributions (Fig. 1). We suggest that the available data are most consistent with an "out of the tropics" (OTT) model, in which the tropics are both a cradle and a museum, with taxa preferentially originating in the tropics and expanding over time into high latitudes without losing their initial tropical distributions. Thus $O_T > O_E$, $E_T \leq E_E$, and $I_T < I_E$.

Until now, direct empirical tests of this model have been lacking, although one biogeographic model suggests that such a dynamic could explain the age-frequency distributions of bivalve genera found in polar oceans today (*35*), and some

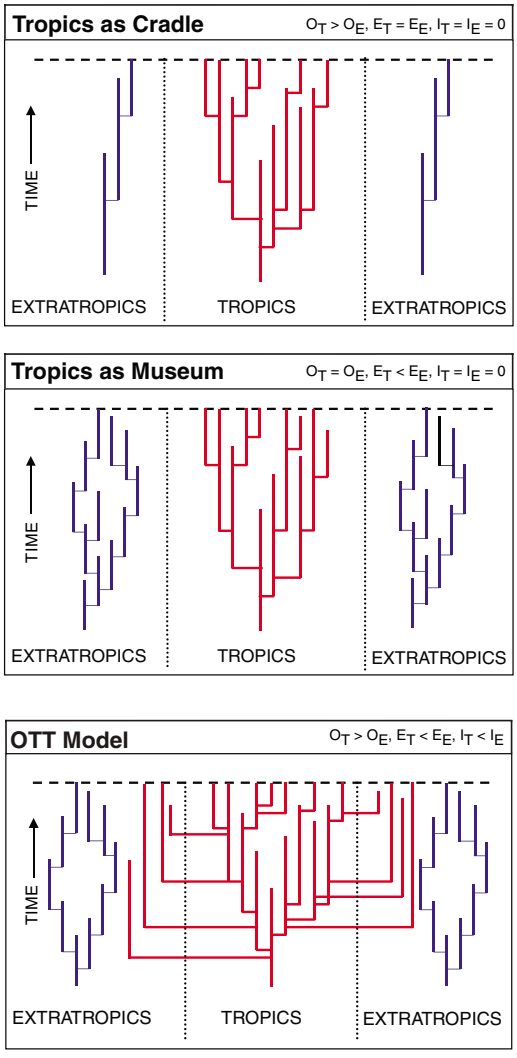


Fig. 1. Simple hypothetical scenarios illustrating the cradle, museum, and OTT models. Red denotes lineages that originated in the tropics; blue denotes lineages that originated outside the tropics. The horizontal lines connecting sister lineages also represent geographic distributions; those extending from tropics to extratropics denote clades that originated in the tropics but have subsequently extended their ranges into extratropical regions while retaining a tropical presence. Many other combinations of these parameters are possible. The dashed horizontal line indicates the present day.

phylogenetic analyses find extratropical taxa to be derived from tropical lineages (*29*, *36*). Here we test the OTT model using paleontological and present-day distributions, and frame testable predictions for groups lacking a good fossil record.

Testing the OTT Model

The marine Bivalvia currently provide one of the few systems that can address each of the OTT predictions directly. As a group, bivalves exhibit a strong LDG, not only for species but also at the level of genera and subgenera (henceforth simply termed genera) (*37*, *38*), which have been the preferred units for large-scale paleontological analyses owing to their taxonomic stability and the robustness of the patterns to sampling artifacts relative to species-level data. The fossil record of marine bivalve genera is rich and densely sampled, with a "pull of the Recent" (the artifact that can arise via strong differences in the sampling of present-day and geologic time intervals) of less than 5% (*39*). Remaining preservational effects are increasingly well understood (*39*–*42*), so that artifacts can be avoided or minimized. Bivalves occur at all latitudes in the modern oceans, and sampling of their fossil record is almost as widespread, although it is not unbiased spatially (*43*).

Taxonomic standardization, a prerequisite for rigorous analysis of the spatial and temporal patterns of biodiversity, although not fully complete, has been undertaken for many late Cenozoic occurrences (*39*, *40*). Accordingly, marine bivalves are becoming a model system for macroecological and macroevolutionary analysis (*40*, *44*, *45*), allowing us to test the predictions of the OTT model with data on the modern latitudinal distributions of bivalve genera, the geologic ages of those taxa relative to their present-day distributions, the spatial pattern of the first occurrences of those taxa, and post-origination changes in their latitudinal range limits.

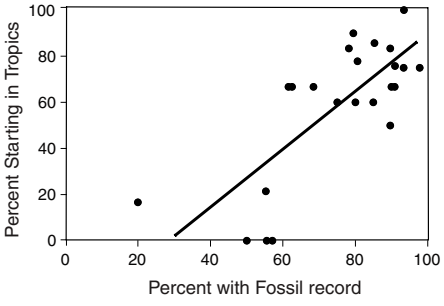


Fig. 2. Sampling quality versus tropical originations in marine bivalve families since the start of the late Miocene (11 million years ago) for families having three or more first occurrences within that interval. Families having more complete fossil records [measured as the proportion of living genera known as fossils (*40*)] tend to show a significantly greater proportion of first occurrences of their constituent taxa in the tropics (simple linear regression, $R^2 = 0.560$, $P = 0.0001$).

$O_T > O_E$. Testing this prediction for genera requires spatially explicit data on their first occurrences in the geologic record, which must be treated cautiously because of biases toward heavier sampling in temperate latitudes (*40*, *43*, *46*–*48*).

One approach to this problem is to use the proportion of living genera known from the fossil record within each bivalve family as a sampling gauge (*49*). For the past 11 million years (from the beginning of the late Miocene to the Recent), the proportion of living taxa that first occur in tropical deposits is positively related to the proportion of taxa known from the fossil record: The better the fossil record of a family, the higher the proportion of its genera that first occurs in the tropics (Fig. 2).

We can also tie a more detailed analysis of the geography of origination to the quality of each family's fossil record (*49*). Restricting analyses to families having ≥75% of their genera known as fossils, tropical first occurrences of those bivalve taxa significantly exceed extratropical ones in each of three successive geologic time intervals leading up to the present day (late Miocene, Pliocene, and Pleistocene; Fig. 3, A, C, and E). Summing over the entire 11-million-year interval, we record 117 tropical and 46 extratropical first occurrences (a significant difference, $P = 2.543 \times 10^{-8}$), indicating that the overall pattern will be robust to any error in the assignment of individual stratigraphic units to our three time bins. And because sampling is strongly biased in the opposite direction (so that some genera originating in the tropics will not be recorded paleontologically until they expand into the better-sampled extratropical zones), these data are

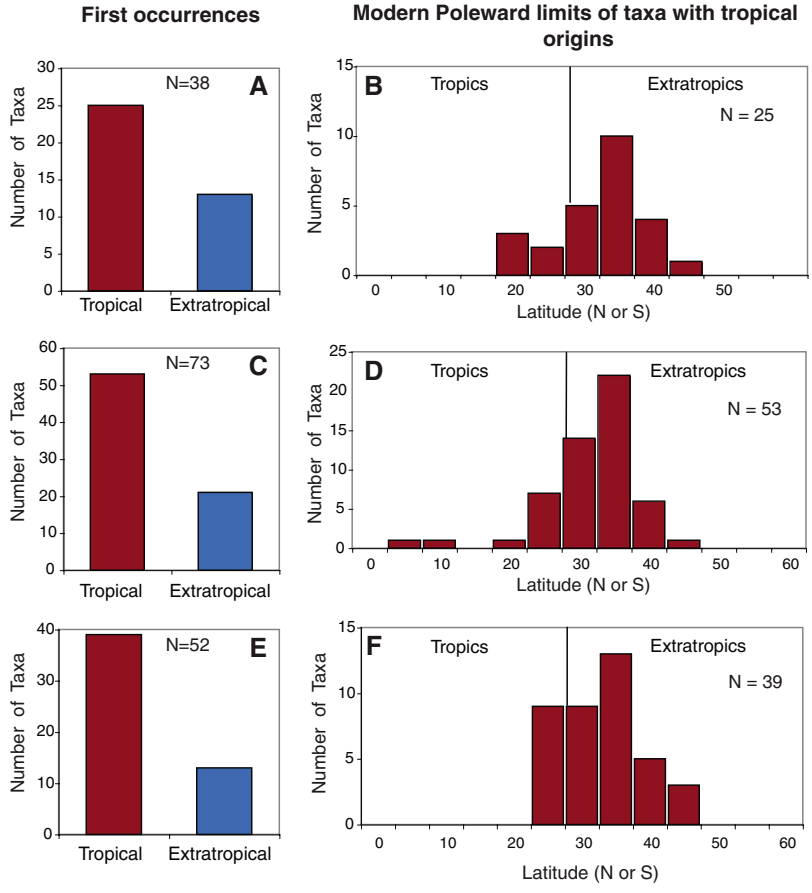


Fig. 3. Latitudinal differences in originations (left) and present-day range limits of marine bivalve genera first occurring in the tropics (right), using only families with ≥75% of their living taxa known as fossils. (**A** and **B**) Genera first appearing in the Pleistocene. (**C** and **D**) Genera first appearing in the Pliocene. (**E** and **F**) Genera first appearing in the late Miocene. *N* indicates the total number of genera in each analysis. For (C) and (E), tropical first occurrences are significantly more frequent than extratropical ones and marginally so for (A), despite the sampling bias favoring extratropical occurrences [(A), $P = 0.07$; (C), $P = 0.0001$; (E), $P = 0.0004$; exact binomial test]. These results are not sensitive to the cutoff value: For example, for the Pliocene, if we use 80% having a fossil record, we find 39 tropical versus 18 extratropical first appearances (FAs); using 70% having a fossil record, we find 52 tropical versus 22 extratropical FAs. Similarly, for the late Miocene, if we use 80% having a fossil record, we find 26 tropical versus 8 extratropical FAs; using 70% having a fossil record, we find 35 tropical versus 9 extratropical FAs. If we treat the data in Fig. 2 as two discrete populations and thus set a 60% cutoff value, we find 38 tropical versus 11 extratropical late Miocene FAs and 56 tropical versus 25 extratropical Pliocene FAs.

almost certainly underestimates of the tropical predominance of first occurrences. The latitudinal difference in originations extends across the Bivalvia and is not just restricted to the heteroconch clade (table S2), which has been the most prolific diversifier through the Cenozoic (*50*).

$E_T \leq E_E$. How extinction rates vary with latitude remains poorly known. Taken at face value, the bivalve data show substantially higher extinctions at high latitudes over the past 11 million years; only 30 exclusively tropical genera go extinct as compared to 107 extratropical and cosmopolitan ones. Factoring in the much greater taxon richness in the tropics suggests an even higher differential in per-taxon rates. These data must again be treated cautiously, owing to the severe undersampling of the tropics, but the presence of so many last occurrences at high latitudes constrains potential patterns and suggests that tropical extinction rates are unlikely to be substantially higher than extratropical ones. These results are also qualitatively consistent with previous studies that have found either little variation in species extinction rates with latitude (*51*) or higher extinction rates of genera and subgenera in polar oceans relative to lower latitudes (*35*). Further analyses of latitudinal trends in extinction rates are needed.

$I_T < I_E$. The bivalve data indicate that genera originating in the tropics tend to extend their ranges to higher latitudes over time, as predicted by the OTT model (*49*). For each of the time bins in Fig. 3, assuming the tropics to be between 25°N and 25°S latitude, ≥75% of the taxa that occur first in the tropics also occur extratropically today; only 2 of those taxa have left the tropics entirely (Fig. 3, B, D, and F; the proportions are >80% if 23° is taken as the edge of the tropics). Again, because the number of taxa known to start in the tropics is undersampled, these values of I_E are almost certainly underestimates.

Insights from Modern Biogeography

The direct tests listed above require temporal and spatial data on ancient distributions that are not available for many important groups of organisms. In such cases, biogeographic data from living taxa can be tested for consistency with the OTT model, although they will not be definitive tests of the model for the reasons outlined above.

Endemism versus latitude. If genera primarily originate in the tropics and expand into extratropical regions, then the simplest biogeographic prediction is that endemism today should decrease with latitude. This prediction is clearly supported for present-day marine bivalves (*49*), in which the LDG persists if we simply exclude all genera restricted to extratropical latitudes: Most of the diversity of extratropical regions comes from taxa shared with the tropics [(*49*) and fig. S1]. However, this is strictly a consistency test, evaluating the tendency of taxa to expand outside of their initial geographic distributions (assuming that each taxon starts with a single species within a single climate zone), without establishing the direction of those expansions.

Age versus latitude. If living genera preferentially originated in the tropics and subsequently expanded into higher latitudes, their average ages should increase with latitude, with the tropics harboring both old and young taxa and higher latitudes progressively lacking in younger taxa. For marine bivalves, both mean and median geologic ages of genera occurring in 10° latitudinal bins increase from the equator to the poles (*49*) (fig. S2), and the age-frequency distributions of tropical and polar assemblages differ significantly (fig. S3). However, such trends suffer from the problem of spatial autocorrelation (the right tails of the histograms in fig. S3 share many taxa) and cannot separate the OTT model from the more traditional "tropics as cradle" hypothesis. A better approach is to test for spatial differences in the shapes of taxon age distributions, derived paleontologically or from well-calibrated molecular phylogenies, against predictions of models that incorporate originations, extinctions, and range expansions of taxa (*35*). Alternatively, reconstructing ancestral geographic ranges of individual taxa from well-supported phylogenies of living species (*52*), in conjunction with biogeographic data, should permit indirect tests. Finally, the finding that the steepest latitudinal gradients occur in the geologically youngest clades of bivalves (*50*, *53*) is also consistent with a dynamic involving preferential origination at low latitudes and poleward expansion over time.

Conclusion

Our goal here has not been to formulate yet another hypothesis about the evolutionary dynamics underlying the LDG; most possible combinations of origination, extinction, and spatial shifts have already been proposed. Instead, we suggest that the long-standing "tropics as cradle or museum" paradigm is not supported by paleontological data or present-day biogeographic patterns [also see (*29*)]. The OTT alternative posits that lineages not only preferentially originate in the tropics but also persist there as they expand poleward; it does not preclude extratropical speciation, of which there are many examples (*54*), but predicts that most extratropical species belong to lineages that originated in the tropics. Thus, the OTT dynamic is likely to be strongest at the level of lineages (for example, genera and families), and we view this model as providing a framework for understanding latitudinal patterns of speciation. Preferential origination of taxa in the tropics followed by range expansion into high latitudes has been proposed on biogeographic and phylogenetic grounds (*34*, *36*, *55*, *56*), and the dynamic is consistent with previous paleontological analyses (*46*). The OTT model is similar to the niche conservatism model (*29*) in that both view the tropics as a cradle and a museum of diversity [see also (*57*, *58*)], but our model differs in emphasizing the expansion of geographic distributions over time; we see "niches" of taxa expanding over time, perhaps as species proliferate within and among climate zones. The general scarcity of robust spatial data on where individual taxa originate has hindered direct tests of these dynamics.

The OTT dynamic documented here suggests that the LDG is shaped by the interaction of two different kinds of processes: those that drive the higher origination rates in the tropics and those that determine the geographic range limits of individual taxa, which makes it difficult to untangle causal mechanisms. We still know little about why taxa preferentially originate at lower latitudes; of the many proposed hypotheses (*46*, *59*–*61*), empirical tests have yielded mixed results for some (*61*–*66*) whereas others remain untested. Similarly, the controls on the geographic range limits of taxa are poorly understood, although theoretical and empirical studies are beginning to address this issue (*67*, *68*). Progress is clearly needed on both fronts, particularly if the source-sink macroevolutionary and biogeographic dynamic outlined here is a general feature of diversity gradients (for example, along bathymetric, elevational, and longitudinal gradients) (*56*, *57*).

The OTT model also has implications for present-day biodiversity, beyond providing a framework for modeling biotic responses to future climate changes. If the tropics are the engine of global biodiversity, as suggested by our analyses (see also table S1), then major losses of tropical taxa will have a global effect by suppressing the primary source of evolutionary novelty for all latitudes. A tropical diversity crisis would thus not only affect tropical biotas but also have profound long-term evolutionary consequences for biotas at higher latitudes.

References and Notes

1. H. Hillebrand, *Am. Nat.* **163**, 192 (2004).
2. L. R. Leighton, *Evol. Ecol.* **19**, 563 (2005).
3. J. A. Crame, *Divers. Distrib.* **7**, 175 (2001).
4. M. L. Rosenzweig, *Species Diversity in Space and Time* (Cambridge Univ. Press, Cambridge, 1995).
5. F. Cecca, *Palaeobiogeography of Marine Fossil Invertebrates—Concepts and Methods* (Taylor and Francis, London, 2002).
6. J. Z. Shen, G. R. Shi, *Palaeogeogr. Palaeoclimatol. Palaeoecol.* **208**, 235 (2004).
7. F. Cecca, B. Vrielynck, T. Lavoyer, H. Gaget, *J. Biogeogr.* **32**, 535 (2005).
8. D. Jablonski, *Paleobiology* **31** (suppl.), 192 (2005).
9. J. R. G. Turner, *Basic Appl. Ecol.* **5**, 435 (2004).
10. B. A. Hawkins, *Trends Ecol. Evol.* **16**, 470 (2001).
11. R. Lewin, *Science* **244**, 527 (1989).
12. B. A. Hawkins *et al.*, *Ecology* **84**, 3105 (2003).
13. M. R. Willig, D. M. Kaufman, R. D. Stevens, *Annu. Rev. Ecol. Syst.* **34**, 273 (2003).
14. D. J. Currie *et al.*, *Ecol. Lett.* **7**, 1121 (2004).
15. M. A. Rex, J. A. Crame, C. T. Stuart, A. Clarke, *Ecology* **86**, 2288 (2005).
16. S. L. Chown, K. J. Gaston, *Trends Ecol. Evol.* **15**, 311 (2000).
17. K. J. Gaston, *Nature* **405**, 220 (2000).
18. G. L. Stebbins, *Flowering Plants: Evolution above the Species Level* (Belknap, Cambridge, MA, 1974).
19. A. R. Wallace, *Tropical Nature and Other Essays* (Macmillan, London, 1878).
20. A. G. Fischer, *Evol. Int. J. Org. Evol.* **14**, 64 (1960).
21. N. C. Stenseth, *Oikos* **43**, 417 (1984).
22. S. Nee, E. C. Holmes, R. M. May, P. H. Harvey, *Philos. Trans. R. Soc. London Ser. B* **344**, 77 (1994).
23. E. Paradis, *J. Theor. Biol.* **229**, 19 (2004).
24. J. A. Todd *et al.*, *Proc. R. Soc. London Ser. B* **269**, 571 (2002).

25. M. Cardillo, *Proc. R. Soc. London Ser. B* **266**, 1221 (1999).
26. M. Cardillo, C. D. L. Orme, I. P. F. Owens, *Ecology* **86**, 2278 (2005).
27. S. T. Jackson, J. W. Williams, *Annu. Rev. Earth Planet. Sci.* **32**, 495 (2004).
28. J. W. Valentine, D. Jablonski, in *Species Diversity in Ecological Eommunities*, R. E. Ricklefs, D. Schluter, Eds. (Univ. of Chicago Press, Chicago, IL, 1993), pp. 341–349.
29. J. J. Wiens, M. J. Donoghue, *Trends Ecol. Evol.* **19**, 639 (2004).
30. A. D. Hecht, B. Agan, *Syst. Zool.* **21**, 308 (1972).
31. J. A. Wiens, *Funct. Ecol.* **3**, 385 (1989).
32. R. E. Ricklefs, *Ecol. Lett.* **7**, 1 (2004).
33. P. J. Darlington, *Evol. Int. J. Org. Evol.* **13**, 488 (1959).
34. K. J. Gaston, T. M. Blackburn, *Proc. R. Soc. London Ser. B* **263**, 63 (1996).
35. E. E. Goldberg, K. Roy, R. Lande, D. Jablonski, *Am. Nat.* **165**, 623 (2005).
36. W. S. Judd, R. W. Sanders, M. J. Donoghue, *Harv. Pap. Bot.* **5**, 1 (1994).
37. C. A. Campbell, J. W. Valentine, *Paleobiology* **3**, 49 (1977).
38. K. Roy, D. Jablonski, J. W. Valentine, *Proc. R. Soc. London Ser. B* **267**, 293 (2000).
39. D. Jablonski, K. Roy, J. W. Valentine, R. M. Price, P. S. Anderson, *Science* **300**, 1133 (2003).
40. J. W. Valentine, D. Jablonski, S. M. Kidwell, K. Roy, *Proc. Natl. Acad. Sci. U.S.A.* **103**, 6599 (2006).
41. S. M. Kidwell, *Science* **307**, 914 (2005).
42. E. M. Harper, in *The Adequacy of the Fossil Record*, S. K. Donovan, C. R. C. Paul, Eds. (Wiley, Chichester, UK, 1998), pp. 243–267.
43. For example, an inventory of published material and the holdings of the Leiden Natural History Museum for the molluscan faunas of Indonesia, the richest and most extensive paleontological sample of the entire Indo-West Pacific core of marine molluscan diversity (*69*), finds 1596 late Miocene and 4532 Pliocene specimens. Extratropical samples for the same interval are far too numerous to list, but five studies out of the many dozens published involve considerably more than 150,000 late Miocene and Pliocene bivalve specimens (*70–74*).
44. D. Jablonski, K. Roy, J. W. Valentine, in *Macroecology; Concepts and Consequences*, T. M. Blackburn, K. J. Gaston, Eds. (Blackwell, Oxford, 2003), pp. 368–390.
45. K. Roy, D. Jablonski, J. W. Valentine, in *Frontiers of Biogeography*, M. V. Lomolino, L. R. Heany, Eds. (Sinauer, Sunderland, MA, 2004), pp. 151–170.
46. D. Jablonski, *Nature* **364**, 142 (1993).
47. J. B. C. Jackson, K. G. Johnson, *Science* **293**, 2401 (2001).
48. P. A. Allison, D. E. G. Briggs, *Geology* **21**, 65 (1993).
49. Materials and methods are available as supporting material on *Science* Online.
50. J. A. Crame, *Paleobiology* **26**, 188 (2000).
51. S. M. Stanley, W. O. Addicott, K. Chinzei, *Geology* **8**, 422 (1980).
52. R. H. Ree, B. R. Moore, C. O. Webb, M. J. Donoghue, *Evol. Int. J. Org. Evol.* **59**, 2299 (2005).
53. J. A. Crame, *Paleobiology* **28**, 184 (2002).
54. D. Schluter, *The Ecology of Adaptive Radiation* (Oxford Univ. Press, Oxford, 2000).
55. R. E. Ricklefs, D. Schluter, in *Species Diversity in Ecological Communities*, R. E. Ricklefs, D. Schluter, Eds. (Univ. of Chicago Press, Chicago, IL, 1993), pp. 350–363.
56. J. C. Briggs, *J. Biogeogr.* **30**, 1 (2003).
57. B. A. Hawkins, J. A. F. Diniz-Filho, S. A. Soeller, *J. Biogeogr.* **32**, 1035 (2005).
58. D. D. McKenna, B. D. Farrell, *Proc. Natl. Acad. Sci. U.S.A.* **103**, 10947 (2006).
59. K. Rohde, *Oikos* **65**, 514 (1992).
60. D. Schemske, in *Ecological and Evolutionary Perspectives on the Origins of Tropical Diversity*, R. Chazdon, T. Whitmore, Eds. (Univ. of Chicago Press, Chicago, IL, 2002), pp. 163–173.
61. A. P. Allen, J. H. Brown, J. F. Gillooly, *Science* **297**, 1545 (2002).
62. S. D. Wright, R. D. Gray, R. C. Gardner, *Evol. Int. J. Org. Evol.* **57**, 2893 (2003).
63. S. S. Pawar, *Evol. Int. J. Org. Evol.* **59**, 234 (2005).
64. J. M. Brown, G. B. Pauly, *Evol. Int. J. Org. Evol.* **59**, 238 (2005).
65. L. Bromham, M. Cardillo, *J. Evol. Biol.* **16**, 200 (2003).
66. P. R. Martin, J. K. McKay, *Evol. Int. J. Org. Evol.* **58**, 938 (2004).
67. T. J. Case, R. D. Holt, M. A. McPeek, T. H. Keitt, *Oikos* **108**, 28 (2005).
68. C. Parmesan *et al.*, *Oikos* **108**, 58 (2005).
69. A. G. Beu, *Scripta Geol.* **130**, 1 (2005).
70. E. Ferrero, B. Merlino, *Boll. Malac.* **28**, 101 (1992).
71. E. Ferrero, B. Merlino, A. Provera, *Boll. Malac.* **33**, 43 (1998).
72. G. Pavia, *Boll. Soc. Paleontol. Ital.* **14**, 99 (1975).
73. S. L. B. Wood, thesis, Virginia Polytechnic Institute, Blacksburg, VA (2006).
74. R. A. Cooper *et al.*, *Geology* **34**, 241 (2006).
75. We thank K. Amano, L. C. Anderson, A. G. Beu, J. S. Crampton, E. V. Coan, T. A. Darragh, H. H. Dijkstra, E. M. Harper, C. S. Hickman, the late K. Lamprell, P. A. Maxwell, P. M. Mikkelsen, P. Middelfart, N. J. Morris, G. Paulay, F. Scarabino, J. A. Schneider, J. D. Taylor, J. D. Todd, T. R. Waller, A. Warén, and F. P. Wesselingh for generously sharing their expertise on bivalve systematics, biogeography, and paleontology; S. K. Donovan and F. P. Wesselingh for access to Leiden Museum collections; P. S. Anderson, G. Hunt, A. J. McGowan, R. M. Price, T. A. Rothfus, R. J. Rundell, and H. J. Sims for assistance in data acquisition and entry; participants in the Gradients in Biodiversity and Speciation Working Group at the National Center for Ecological Analysis and Synthesis for insightful discussions; and S. M. Kidwell and three anonymous reviewers for valuable comments. Supported by a grant from NASA.

Supporting Online Material

www.sciencemag.org/cgi/content/full/314/5796/102/DC1
Materials and Methods
Figs. S1 to S3
Tables S1 and S2
References

5 June 2006; accepted 9 August 2006
10.1126/science.1130880

REPORTS

Molecular Loops in the Galactic Center: Evidence for Magnetic Flotation

Yasuo Fukui,[1]* Hiroaki Yamamoto,[1] Motosuji Fujishita,[1] Natsuko Kudo,[1] Kazufumi Torii,[1] Satoshi Nozawa,[2] Kunio Takahashi,[3,4] Ryoji Matsumoto,[5] Mami Machida,[3] Akiko Kawamura,[1] Yoshinori Yonekura,[6] Norikazu Mizuno,[1] Toshikazu Onishi,[1] Akira Mizuno[7]

The central few hundred parsecs of the Milky Way host a massive black hole and exhibit very violent gas motion and high temperatures in molecular gas. The origin of these properties has been a mystery for the past four decades. Wide-field imaging of the ^{12}CO (rotational quantum number $J = 1$ to 0) 2.6-millimeter spectrum has revealed huge loops of dense molecular gas with strong velocity dispersions in the galactic center. We present a magnetic flotation model to explain that the formation of the loops is due to magnetic buoyancy caused by the Parker instability. The model has the potential to offer a coherent explanation for the origin of the violent motion and extensive heating of the molecular gas in the galactic center.

The magnetic field in the central hundred parsecs of the Milky Way is substantially stronger than elsewhere in the Galaxy, at least in the prominent nonthermal features emitted from high-energy electrons spiraling along magnetic field lines. The magnetic field of these electrons is estimated to be typically a milligauss (*1*, *2*), although some recent works suggest a weaker global magnetic field in the galactic center (*3*). Magnetic fields have the potential to affect the dynamics of molecular gas and may control star formation on a small scale and govern the motion of molecular clouds on a large scale. An observational link between the molecular gas and the magnetic field in the galactic center has been obtained through polarization measurements of magnetically aligned dust grains at mid- to far-infrared to submillimeter wavelengths (*4*, *5*).

Here, we report millimeter-wave observations of two molecular features that have a loop-like shape with a length of several hundred parsecs and width of ~30 pc within ~1 kpc

[1]Department of Astrophysics, Nagoya University, Chikusa-ku, Nagoya 464-8602, Japan. [2]Department of Science, Ibaraki University, 2-1-1 Bunkyo, Mito, Ibaraki 310-8512, Japan. [3]National Astronomical Observatory of Japan, 2-21-1 Osawa, Mitaka, Tokyo 181-8588, Japan. [4]The Graduate University for Advanced Studies, Hayama, Miura, Kanagawa, 240-0193, Japan. [5]Department of Physics, Faculty of Science, Chiba University, 1-33, Yayoi-cho, Inage-ku, Chiba 263-8522, Japan. [6]Department of Physical Science, Osaka Prefecture University, 1-1 Gakuen-cho, Sakai, Osaka 599-8531, Japan. [7]Solar-Terrestrial Environment Laboratory, Nagoya University, Chikusa-ku, Nagoya, 464-8601, Japan.

*To whom correspondence should be addressed. E-mail: fukui@a.phys.nagoya-u.ac.jp

from the center. We interpret these features as magnetically floated loops within the nuclear disk, analogous to the solar loops, caused by the Parker instability.

The new molecular image of the 2.6-mm CO emission obtained with NANTEN, a millimeter/submillimeter telescope with a 4-m diameter, is shown in Fig. 1 (*6*, *7*). The CO intensity distribution at a negative radial velocity referred to the local standard of rest—defined as a point in space that has a velocity equal to the average velocity of all stars including the Sun located within 100 pc of the Sun (hereafter referred to as velocity)—reveals two loops named here as loop 1 and loop 2 (Fig. 1, A and B). In a galactic latitude range from $b = 0°$ to 2°, loop 1 and loop 2 are seen at $l \approx 356°$ to 358° and $l \approx$ 355° to 356°, respectively. The typical widths of the loops are ~0.2°.

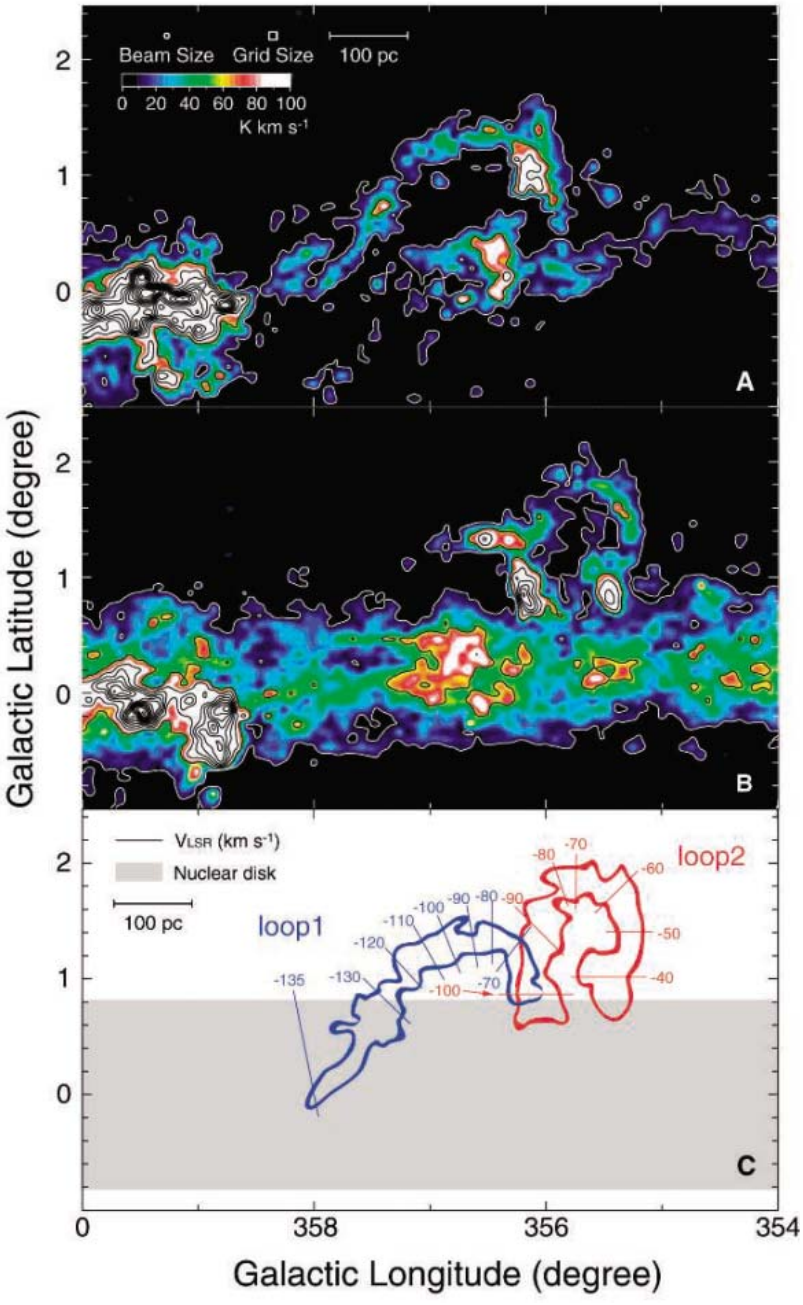


Fig. 1. Integrated intensity map of loops 1 and 2 in ^{12}CO (J = 1 to 0) 2.6-mm wavelength emission. (**A**) Loop 1 is shown toward $l \approx 356°$ to 358°. The integration range in velocity is from −180 to −90 km s^{-1}. Contours are illustrated from 7.1 K km s^{-1} (white) with an interval of 50 K km s^{-1} (black). (**B**) Loop 2 is shown toward $l \approx 355°$ to 356°. The region is the same as that in (A), but the integration range in velocity is from −90 to −40 km s^{-1}. Contours and color scale are the same as in (A). The feature around (l, b) = (356.53°, 1.33°) corresponds to loop 1. (**C**) Schematic drawing of loop 1 (blue) and loop 2 (red). Numbers indicate the velocity in the loop in kilometers per second by averaging the several pixels nearby. Local standard of rest (V_{LSR}) is defined as a point in space that has a velocity equal to the average velocity of all stars including the Sun with 100 pc of the Sun.

The projected velocity distribution within the loops is shown in two ways. First, the average velocity in the loops is shown in Fig. 1C. Second, a longitude-velocity diagram presents the two loops in Fig. 2. The data in Fig. 2 indicate that the two loops have strong velocity gradients: ~80 km s^{-1} per 250 pc along loop 1 and ~60 km s^{-1} per 150 pc along loop2.

It is clear that the foot points of the loops at $b \approx 0.7°$ show a very broad linewidth of ~40 to 80 km s^{-1}, as seen at $l \approx 355.5°$, 356°, and 357.4° [Fig. 2, supporting online material (SOM) text, and figs. S3 and S4]. The broadest one, at $l \approx 356°$, is actually a superposition of two components at $l \approx 356.20°$ and 356.05°; the foot points of loop 1 and loop 2 are evident in the optically thin ^{13}CO data (Fig. 2). These large velocity spans in the foot points of the loops are characteristic of the molecular gas near the galactic center, including the so-called central molecular zone (CMZ) with a radius of a few hundred parsecs (*2*, *8*, *9*), and are quite unusual in the disk molecular clouds that have much smaller velocity extents of less than 10 km s^{-1}. Therefore, we classified the loops as being located in the galactic center, and we adopt a distance of 8.5 kpc, hereafter. The projected lengths of the loops were then estimated as ~500 and ~300 pc for loops 1 and 2, respectively, with typical widths of ~30 pc. The heights of these loops are ~220 to 300 pc from the galactic plane, substantially higher than the typical scale height in the nuclear disk of ~100 pc (SOM text and fig. S5).

By combining the ^{12}CO and ^{13}CO data and assuming local thermodynamical equilibrium at 50 K, we estimated that the total molecular mass of loops 1 and 2 was ~1.7×10^5 solar masses as a conservative lower limit and that each of the loops has a total mass of ~0.8×10^5 solar masses (SOM text). The kinetic energy involved in a loop was estimated to be ~0.9×10^{51} erg for a velocity dispersion of 30 km s^{-1}. This energy is too large to be explained by a single supernova explosion if we take into account that only a part of the explosion energy of a supernova, 10^{51} erg, can be converted into the gas kinetic energy; it is also too large to be explained by a supershell if we take into account that the maximum velocity extents of CO in a supershell is only ~20 km s^{-1} (*10*, *11*), much smaller than the present ones, which are ~80 km s^{-1}. In addition, an expanding shell would look like a circle in the position-velocity diagram instead of the linear features we observed (Fig. 2).

We now present a model incorporating the Parker instability in an effort to explain the formation of the two loops (*12*, *13*). The Parker instability is a magnetohydrodynamic instability that occurs if a gas layer is supported in part

by horizontal magnetic fields against a gravitational field. In this instability, the gas layer will begin to undulate when it is disturbed in space. Linear theory and nonlinear numerical simulations have been extensively developed on the instability in such a gas disk with frozen-in magnetic fields (*14*, *15*). We assumed that the initial state is a nuclear gas disk of ~1.3-kpc radius consisting of atomic and molecular gas, for which only the azimuthal toroidal field components were assumed (*16*). The molecular part of the disk, whose dominant component is CMZ with a radius of a few hundred parsecs, is likely ionized at an ionization degree of $\sim 10^{-7}$, which allows the frozen-in condition—i.e., the complete dynamical coupling of the molecular gas to the magnetic field (*17*). We assumed that the instability takes place in the disk to float the gas as a loop that has a nearly parallel or helical magnetic field. In the case of the loops we observed, this flotation may be rapid, and the gas inside the loop can become gravitationally attracted to the plane during the flotation. This process obviously accelerates the molecular gas along the field lines downward, and the highest velocities—nearly the Alfvén speed—are achieved near the foot points of the loops to form shock fronts on the disk surface (*13*).

The key parameters in the magnetic flotation are the pressure scale height, H, and the Alfvén speed $V_A = B/\sqrt{4\pi\rho}$, where B is the magnetic field and ρ is the gas density. By assuming a magnetic pressure-dominated disk, the minimum growth time of the loop is given as several times larger than the Alfvén transit time over one scale height, and the most unstable wavelength of a floated loop is given as about 10 times the scale height in the linear theory (*18*). We assumed energy equi-partition between gas motion and magnetic field and estimated the equi-partition of the magnetic field $B_{eq} \sim 5 \times 10^{-12}\langle n\rangle^{0.5} V_t$ (gauss) ~ 150 μG at $R \sim 500$ pc, where mean density is $\langle n\rangle \sim 100$ cm^{-3} and turbulent velocity $V_t \sim 30$ km s^{-1}, much weaker than the milligauss field for CMZ (*19*). This value does not contradict 3σ upper limits for a line-of-sight component of the magnetic field of ~300 μG from OH Zeeman measurements at about R of the loops, where R is the radius from the galactic center (*20*). By taking this field, we estimated Alfvén velocity, V_A, as ~ 24 km s^{-1} for typical molecular hydrogen number density in the loops of ~100 cm^{-2}. Taking a scale height of ~100 pc at $R \sim 500$ pc, we then predicted that the loop has a growth time scale of several millions of years and a length projected on the disk of ~ 1 kpc. The flowing velocity in the loop is estimated to be comparable to V_A on the disk. We found that these first-order estimates of the basic observed quantities agree well with the observations of the loops.

Furthermore, the nonlinear numerical simulations indicated the generation of shock fronts at the foot points of the loop and nearly vertical elongated distribution of dense gas as a result of down flow toward the plane (*13*). The very broad velocity features of ~40 to 80 km s^{-1}, narrowly constrained within several arc minutes toward $l \approx 355.5°$, 356°, and 357.4° (Fig. 2), are indeed consistent with such predicted shocks at the foot points of the loops. Molecular gas guided by the magnetic loop moves toward both ends and an apparent velocity gradient should be observed along the loop; such gradients are seen in Figs. 1 and 2.

By assuming that the plane including the loops is perpendicular to the disk, we found that two model loops calculated by a magnetohydrodynamical code were a good match for the observations after appropriate tuning of three model parameters: the tilt of the loops to the line of sight, the magnitude of the velocity, and the time elapsed since the beginning of the flotation within the ranges given theoretically (*7*) (Fig. 3 and figs. S1 and S2). When the fits were made, loop 1 was located on the near side of the disk with an approximate inclination angle to the line of sight of ~40°, and loop 2 was also on the near side with a larger inclination angle of ~20°. In these geometries, the maximum velocities in the loops were ~30 to 50 km s^{-1} relative to their velocity centroid. The apparent sizes of the loops along the longitude were also consistent with the projection of a wavelength of a loop (~1 kpc).

Among the mysterious features unique to the galactic center, the very violent motion in CMZ and the other similar broad velocity features whose velocity dispersion is ~15 to 30 km s^{-1} have been highly enigmatic in the past few decades; another mysterious feature has been the extensive distribution of warm molecular gas of ~70 to 300 K as derived in the spectra of CO, NH_3, H_2, H_3^+, etc. (*21*–*23*), whose heating source has not yet been identified (*2*, *17*). The most salient three broad features at $l \approx 1.3°$, 3°, and 5° known to date (*8*, *24*, *25*) that have

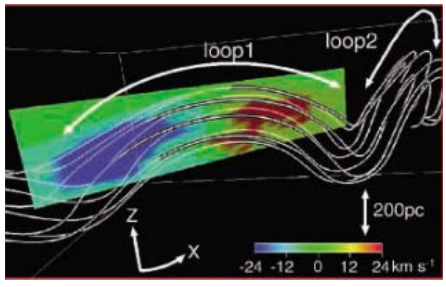


Fig. 3. Image projected on the sky based on a typical two-dimensional (2D) calculation of the Parker instability in a magnetized gas disk displayed in 3D. The image represents the last stage of the simulation at ~9 ×10^6 years since the beginning of the flotation of the two loops. White lines and color scale show the magnetic field lines of the two loops and the velocity along loop 1, respectively, under the situation given in the text. We used the parameters of (H, V_A) = (100 pc, 24 km s^{-1}) derived from the observations in this simulation, where H and V_A are the pressure scale height in the disk and the Alfvén speed, respectively. The magnetic field is taken to be 150 μG by assuming that the number density of hydrogen, temperature, and plasma beta (gas pressure/magnetic pressure) are 100 cm^{-3}, 100 K, and 0.2, respectively. Our result is valid for any values of the concerned parameters because our model is nondimensional and scale-free. Nondimensional magneto-hydrodynamic (MHD) equations are solved numerically with the use of a modified Lax-Wendroff scheme and the artificial viscosity used in previous studies (*6*, *13*, *26*).

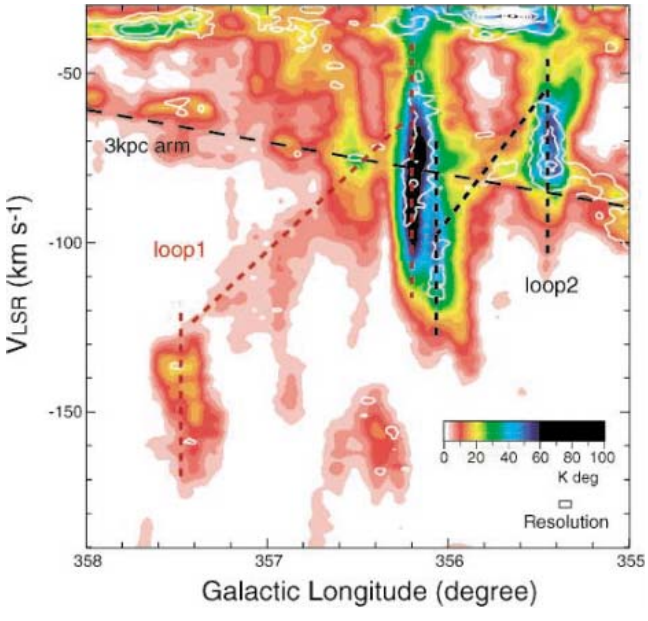


Fig. 2. Velocity-galactic longitude diagram toward loops 1 and 2 in $J = 1$ to 0 ^{12}CO 2.6-mm (color) and ^{13}CO 2.7-mm (contours) emission. The integration range in the galactic latitude is from 0.5° to 2.0° in ^{12}CO and from 0.5° to 1.0° in ^{13}CO. The velocity is smoothed at a resolution of 2 km s^{-1}. The dotted lines show the location of loop 1 (brown) and loop 2 (black). The very broad features at $l \approx 355.5°$, 356°, and 357.4° correspond to foot points of the loops. The ^{13}CO contours are also overlaid to show that the broad one at $l \approx 356°$ consists of two components with ~0.2° shift in the galactic longitude, the foot points of the loops 1 and 2. Contours are illustrated from 1.5 K deg with an interval of 2.0 K deg. The dashed line indicates the 3-kpc arm. The ^{13}CO data are taken for $b < 1°$ in the similar observing parameters by NANTEN.

similar velocity widths of ~100 km s^{-1} exhibit vertical elongations of more than 200 pc with thin widths of 30 pc. These vertical features are consistent with the foot points of a magnetic loop in its late stage of time evolution (*13*). The NANTEN data set indicates that a weak loop-like feature that has a large velocity gradient is "bridging" these vertical features at higher latitudes between $l \approx 3°$ and 5°, suggesting the existence of another magnetic loop in the positive longitude (SOM text and fig. S6). In addition, the model offers naturally substantial heating of the warm molecular gas at the foot points; the velocity dispersion (~15 to 30 km s^{-1}) of the broad CO features corresponds to kinetic temperature higher than about 10^4 K if the shock is completely converted into thermal energy at the foot points. We suggest, therefore, that the present model has the potential to be applied to the other salient broad velocity features in the galactic center and to the heating of the molecular gas at their foot points.

The present model shares the common physics of solar loops. It has been well understood that solar loops are the results of magnetic flotation driven by the motion of the magnetic field lines anchored onto the solar granules (*26*). The size scale of the solar loops is ~12 orders of magnitude smaller than those in the galactic center, and this is a natural consequence of the scale height on the solar surface, only ~200 km, which determines the height of the loop. Despite the large difference in size, the Alfvén speed is ~10 km s^{-1} as calculated from the density (~2.5×10^{-7} g cm^{-3}) and the magnetic field strength (~500 G) on the solar surface (*26*). The small size of the solar loop determines the much shorter time scale of the solar flotation as ~10^3 s, as compared with ~10^{14} s in the galactic center loops.

References and Notes

1. F. Yusef-Zadeh, M. Morris, D. Chance, *Nature* **310**, 557 (1984).
2. M. Morris, E. Serabyn, *Annu. Rev. Astron. Astrophys.* **34**, 645 (1996).
3. T. N. LaRosa *et al.*, *Astrophys. J.* **626**, L23 (2005).
4. D. T. Chuss *et al.*, *Astrophys. J.* **599**, 1116 (2003).
5. M. Morris *et al.*, *Astrophys. J.* **399**, L63 (1992).
6. A. Mizuno, Y. Fukui, *The Structure and Evolution of Our Galaxy*, D. Clemens, R. Shah, T. Brained, Eds. (ASP Conf. Ser. No. 317, ASP, Boston, MA, 2004), pp. 59–65.
7. Materials and methods are available as supporting material on *Science* Online.
8. T. Oka, T. Hasegawa, F. Sato, M. Tsuboi, A. Miyazaki, *Astrophys. J. Suppl. Ser.* **118**, 455 (1998).
9. M. Tsuboi, T. Handa, N. Ukita, *Astrophys. J. Suppl. Ser.* **120**, 1 (1999).
10. Y. Fukui *et al.*, *Publ. Astron. Soc. Japan* **51**, 751 (1999).
11. K. Matsunaga *et al.*, *Publ. Astron. Soc. Japan* **53**, 1003 (2001).
12. E. N. Parker, *Astrophys. J.* **145**, 811 (1966).
13. R. Matsumoto, T. Horiuchi, K. Shibata, T. Hanawa, *Publ. Astron. Soc. Japan* **40**, 171 (1988).
14. K. Shibata, R. Matsumoto, *Nature* **353**, 633 (1991).
15. M. Machida, M. R. Hayashi, R. Matsumoto, *Astrophys. J.* **532**, L67 (2000).
16. W. B. Burton, H. S. Liszt, *Astrophys. J.* **225**, 815 (1978).
17. R. Güsten, S. D. Philipp, in *Proceedings of the Fourth Cologne-Bonn-Zermatt Symposium*, Zermatt, Switzerland, 22 to 26 September 2003, S. Pfalzner, C. Kramer, C. Staubmeier. A. Heithausen, Eds. (Springer, Heidelberg, Germany, 2004), pp. 253–263.
18. T. Horiuchi, R. Matsumoto, T. Hanawa, K. Shibata, *Publ. Astron. Soc. Jpn.* **40**, 147 (1988).
19. J. Bally, A. A. Stark, R. W. Wilson, C. Henkel, *Astrophys. J.* **324**, 223 (1988).
20. K. I. Uchida, R. Güsten, *Astron. Astrophys.* **298**, 473 (1995).
21. T. Oka, G. R. Thomas, M. Goto, T. Usuda, J. B. McCall, *Astrophys. J.* **632**, 882 (2005).
22. I. A. Harris, T. D. Jaffe, M. Silber, R. Genzel, *Astrophys. J.* **294**, L93 (1985).
23. J. N. Rodríguez-Fernández *et al.*, *Astron. Astrophys.* **365**, 174 (2001).
24. M. T. Bania, *Astrophys. J.* **216**, 381 (1977).
25. M. Bitran, H. Alvarez, L. Bronfman, J. May, P. Thaddeus, *Astrophys. J. Suppl. Ser.* **125**, 99 (1997).
26. S. Nozawa, *Publ. Astron. Soc. Jpn.* **57**, 995 (2005).

27. We thank all of the members of the laboratory who contributed to these observations at Las Campanas Observatory (LCO). We appreciate the hospitality of all the staff members of LCO of the Carnegie Institution of Washington (CIW). This project is based on a mutual agreement between Nagoya University and the CIW. We also acknowledge that the operation of NANTEN is made possible by contributions from many Japanese public donators and companies. This work was financially supported in part by Grants-in-Aid for Scientific Research from the Ministry of Education, Science, Sports, and Culture of Japan and from Japan Society for the Promotion of Science.

Supporting Online Material

www.sciencemag.org/cgi/content/full/314/5796/106/DC1
Materials and Methods
SOM Text
Figs. S1 to S6
References

24 May 2006; accepted 14 August 2006
10.1126/science.1130425

Extending Top-Down Mass Spectrometry to Proteins with Masses Greater Than 200 Kilodaltons

Xuemei Han,[1]* Mi Jin,[1]* Kathrin Breuker,[2] Fred W. McLafferty[1]†

For characterization of sequence and posttranslational modifications, molecular and fragment ion mass data from ionizing and dissociating a protein in the mass spectrometer are far more specific than are masses of peptides from the protein's digestion. We extend the ~500-residue, ~50-kilodalton (kD) dissociation limitation of this top-down methodology by using electrospray additives, heated vaporization, and separate noncovalent and covalent bond dissociation. This process can cleave 287 interresidue bonds in the termini of a 1314-residue (144-kD) protein, specify previously unidentified disulfide bonds between 8 of 27 cysteines in a 1714-residue (200-kD) protein, and correct sequence predictions in two proteins, one with 2153 residues (229 kD).

Identifying a protein and characterizing its structure from its mass and masses of its backbone fragments are major proteomics research capabilities. The "top-down" methodology (*1*) analyzes intact proteins by combining electrospray ionization (ESI) (*2*) with high-performance mass spectrometry (MS) such as Fourier transform (FT) MS (*3*). Five to 10 accurate mass values of backbone fragment ions from dissociation of an ionized protein are sufficient to identify it from among the many thousands predicted by the DNA sequence (*4*, *5*). However, the largest molecular ions that have yielded interresidue cleavages contain ~580 and 663 residues (*6*, *7*), with molecular sizes of 67 and 74 kD, respectively. Although no molecular weight (M_w) limitation exists for the "bottom-up" approach (*8–10*) that initially digests the protein into small peptides, for identification of proteins (*5*) top-down MS gives fragments that cover a much larger range of mass values, and these can be produced from a single protein's M_w-selected molecular ions.

The dominant proteomics application of top-down MS has been for the characterization of posttranslational modifications and sequence errors (*1*, *4–7*, *11*). For bottom-up MS, the measured peptide mass values are matched against those of the peptides expected from the DNA-predicted proteins that are unmodified; to detect a modification, matching must also be done against the predicted peptide masses adjusted separately for all likely modifications. Further, bottom-up MS usually achieves only 40 to 90% sequence coverage (*8–10*). For top-down MS, in contrast, a discrepancy between the measured M_w value and that predicted by the DNA sequence directly shows the presence of a modification(s) or sequence error(s). The location of the modification(s) in the protein is then indicated by a corresponding discrepancy(s) in

[1]Department of Chemistry and Chemical Biology, Baker Laboratory, Cornell University, Ithaca, NY 14853, USA. [2]Institute of Organic Chemistry and Center for Molecular Biosciences Innsbruck (CMBI), University of Innsbruck, Innrain 52a, 6020 Innsbruck, Austria.

*These authors contributed equally to this work.
†To whom correspondence should be addressed. E-mail: fwm5@cornell.edu

the fragment ion mass values (*1*, *4*–*7*, *11*). If, in an unknown protein, the measured fragment masses represent random backbone cleavages at 10% of the interresidue sites, it should be possible to locate a single protein modification to within 10 residues, on average. Mass spectra of the 76-residue ubiquitin protein (8.6 kD) gave fragment ions representing cleavage of all 75 interresidue bonds, making possible its de novo sequencing (*12*), but the largest (~580- and 663-residue) protein ions previously dissociated gave <20 cleavages each (*6*, *7*).

For proteins larger than ~500 residues, the intractability for top-down MS appears to result from the increasing complexity of the gaseous molecular ion's tertiary conformer structure (*13*–*17*). Solvent removal from a denatured protein during ESI permits (*13*–*17*) much stronger electrostatic intramolecular interactions such as hydrogen bonds and salt bridges by removing the competition from water, although this process also weakens hydrophobic interactions. Immediately after desolvation of ubiquitin ions electrosprayed from native solution, they were found (*17*) to undergo almost no nozzle-skimmer dissociation (*18*), presumably from fast folding of the nascent gaseous ions. This unusual ion stabilization could be greatly reduced and extensive fragment mass data obtained by the immediate addition of energy in several ways to the newly formed ions (*17*). Here this "prefolding dissociation" (PFD) (Fig. 1) and a method for conformer disruption that involves ESI solution additives are applied to large proteins, achieving cleavage of 108, 287, 87, and 62 interresidue bonds in the termini of 1023-, 1314-, 1714-, and 2153-residue proteins, respectively. A single PFD spectrum, consuming <1 pmol of protein (*19*), defines as many as 100 cleavages.

This intractability of large proteins with conventional ion-dissociation methods was reinvestigated with the protein β-galactosidase (β-Gal; 1023 amino acids, 116 kD) (*21*). After its molecular ions were trapped in the FTMS cell, they were subjected to infrared multiphoton dissociation (IRMPD) (*17*, *22*) under strenuous conditions (80-ms, 27-W CO_2 laser) that resulted in 29 interresidue cleavages (Fig. 2A). However, these conditions produced no cleavages from the three larger proteins studied here. The alternative PFD delays the postulated rapid formation of intramolecular noncovalent bonds both by (Fig. 1) heating the ESI inlet capillary (temperature, T_{cap}) (*16*) and by collisional activation (acceleration by voltage V_{pre}) in the subsequent preskimmer region (~1-Torr pressure, multiple low-energy collisions). Next, collisions (acceleration separately by V_{post}) in the following ~10^{-3}-Torr postskimmer region are of higher energy (far longer mean free path) and thus provide more efficient cleavage of the stronger covalent backbone bonds of the protein ions (*17*). However, dissociation is effected before a specific molecular ion mass can be separated; precursor ion selection is required for MS/MS of mixture samples. Purified proteins and their binary mixtures were examined here.

Experimental variables of the new PFD methods were investigated first with the linear formylglycinamide ribonucleotide amidotransferase (PurL), predicted to have 1315 residues (*23*). Its ESI mass spectrum gave M_w = 143,500 ± 23, inconsistent with the DNA-sequence predicted value of 143,635. Investigating the effect of capillary temperature, T_{cap} = 45°C, gave no backbone cleavages for very vigorous subsequent collision

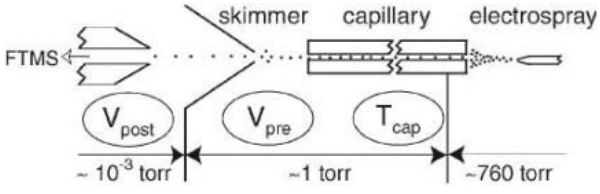


Fig. 1. Prefolding dissociation. Protein solution was electrosprayed from the right into the MS entrance capillary, then heated for ion desolvation and folding retardation; the ions were accelerated by V_{pre} through the preskimmer collision region of short mean-free path to cleave weak noncovalent bonds. Ions were then accelerated by V_{post} through the postskimmer region of long mean-free path to cleave strong backbone covalent bonds.

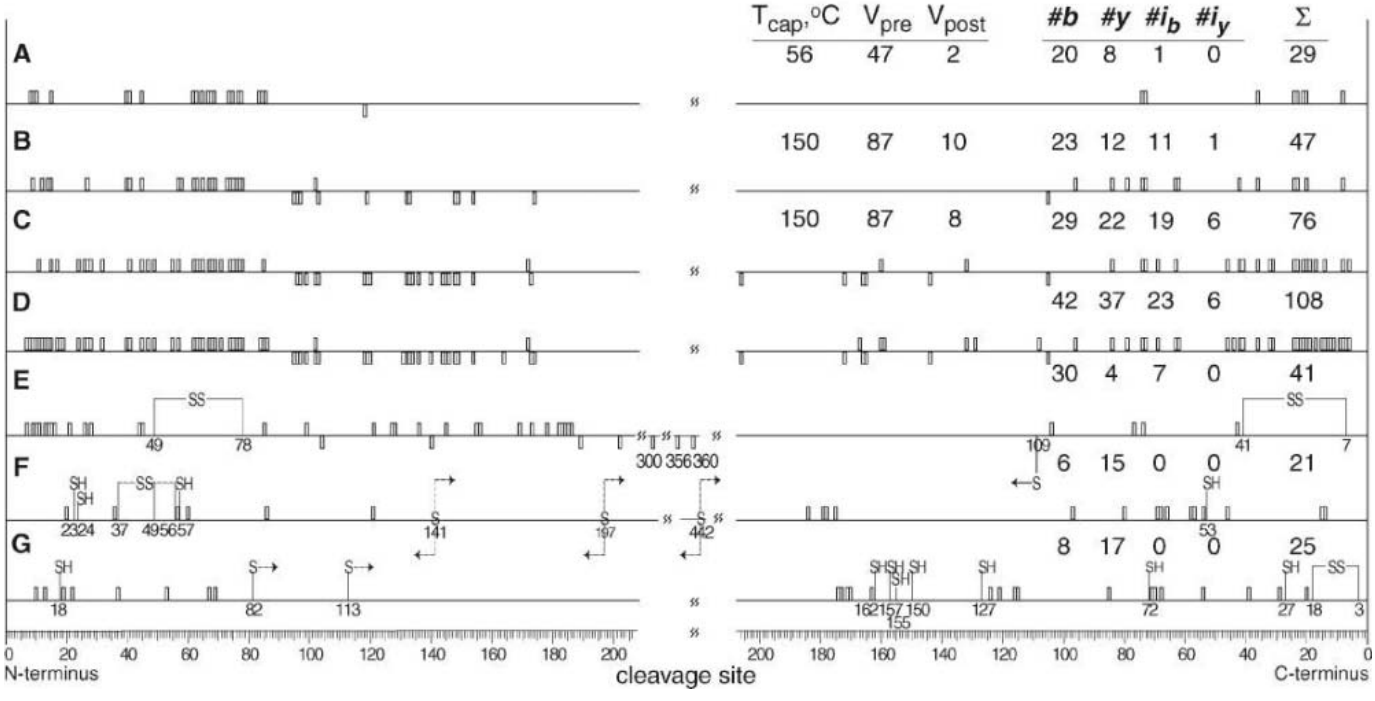


Fig. 2. (**A**) IRMPD mass spectrum of β-Gal (1023 residues). PFD mass spectra of (**B** to **D**) β-Gal [(B), single spectrum without and (C) with 500 μM ammonium tartrate and (D), sum of 17 spectra] and (**E**) β (656 residues), (**F**) α (767 residues), and (**G**) γ (291 residues) chains of human complement C4. Open vertical bars show backbone cleavages. Above the line: left, b ions; right, y ions; below the line: left, i_b ions; right, i_y ions. For (E) to (G), the cysteines (numbered below the line from each terminus) that were found to be unsubstituted are indicated by a single vertical bar topped with –SH; other Cys residues are part of S-S bonds, with a solid line for the S-S bond indicating the identification of both Cys residues. The S-S bond in the α chain is at 37-49, 37-56, or 49-56. Column captions are defined in Table 1.

conditions (V_{pre} = 170 and V_{post} = 18); even increasing T_{cap} to 90°C gave no cleavages at V_{pre} = 125 and V_{post} = 13, but increasing V_{pre} to 145 yielded extensive product ions (Table 1). Of these mass values, 27 could be assigned to the predicted C-terminal sequence; the 23 other mass values were made assignable by removal of the predicted N-terminal methionine to give a revised M_w value of 143,504 versus 143,500 observed (1314 residues). Additional PFD spectra obtained at T_{cap} = 150° (Fig. 3A), 190°, 295°, and 345°C, but with lower collision energies (V_{pre} = 134 to 49 V and V_{post} = 8 to 2 V), each provided 9 to 15 "unique" cleavages (those not found in the other spectra), for a total of 126 different cleavages. At T_{cap} = 190°C, three additional spectra in which V_{pre} was decreased from 133 to 100 V while V_{post} was increased from 5 to 13 V each yielded 9 to 17 unique cleavages, which increased the total number of different 190°C cleavages from 60 to 94. One and two additional T_{cap} = 150° and 295°C spectra, respectively, increased these different cleavages to 100 and 91. These 11 PFD spectra (Fig. 3B) designated a total of 173 different cleavage sites. Increasing the activation by any of the three parameters, but particularly by V_{post}, increased the relative intensity of ions of less than 50 residues, $I_{<50aa}$ (Table 1).

We also tried a variety of small molecules added to the ESI solution to reduce this molecular-ion intractability. Glycerol, ribose, dimethyl sulfoxide, 1,4-cyclohexanedione, and ammonium benzoate gave no appreciable fragmentation improvement or had a negative effect, nor did prior 6 M urea denaturation or 80°C heating of the protein solution in the ESI tip. However, the ammonium salts of citrate, succinate, and tartrate (500 μM; Fig. 3, C to E) increased the interresidue cleavages of PurL in a single spectrum to as many as 100. These additives also produced a more stable electrospray but did not appreciably change the charge-state distribution of these molecular ions and those of cytochrome c or the abundance of background singly charged ions (*19*). Four spectra with the ammonium tartrate additive under a variety of conditions (T_{cap} = 150°C, V_{pre} = 100 to 140 V, V_{post} = 5 to 12 V) gave 174 different cleavages (Fig. 3F), and 10 spectra (T_{cap} = 150° to 190°C, V_{pre} = 65 to 140 V, V_{post} = 3 to 12 V) with additives gave 249 cleavages. All 21 spectra gave 287 different cleavages (Fig. 3G) that represent 73% of the first 100 N- and C-terminal interresidue bonds and 64% of the second 100. Such coverage should make extensive de novo sequence information possible for unknown proteins (*12*). The apparent "ball of spaghetti" tertiary structure only allows for cleavages in its unraveled ends, with >800 central residues untouched despite these multiple activations.

The ESI spectrum of the single-chain protein β-Gal (*21*) gave M_w = 116,355 ± 12 versus 116,352 predicted. PFD at intermediate energies gave 47 different cleavages (Fig. 2B). Added ammonium tartrate increased this number to 76 (Fig. 2C), whereas 17 spectra run under a variety of conditions gave 108 different cleavages (Fig. 2D). These included all 29 cleavages from IRMPD in the FTMS cell (Fig. 2A).

Human complement C4 glycoprotein (1714 residues, M_w = 200,000 by SDS–polyacrylamide gel electrophoresis) consists of three chains linked by three disulfide bonds, but the locations of these or any other S-S bonds of

Table 1. Effect of excitation parameters on PFD mass spectra of PurL (1314 residues). Capillary temperature (T_{cap}) and pre- and postskimmer collision voltages (V_{pre}, V_{post}) versus the number of N- and C-terminal (b, y) and secondary internal (i_b, i_y) product ions, the relative intensity (I) of these ions of <50 amino acids ($I_{<50aa}$), and the total number of different backbone cleavages (Σ), including cleavages unique (Σ_{uniq}) among other values of T_{cap} or of V_{pre} and V_{post}.

T_{cap} (°C)	V_{pre}	V_{post}	#b	#y	#i_b	#i_y	$I_{<50aa}$ (%)	Σ	Σ_{uniq}
90	145	13	11	17	12	10	49	50	15*
150	134	5	24	16	17	10	50	67	13*
295	60	8	23	17	12	17	36	69	12*
345	49	2	22	15	9	9	41	55	9*
190	133	5	24	14	15	7	36	60	17†‡
190	108	5	15	12	9	6	29	42	10†
190	105	8	15	13	10	8	49	46	9†
190	100	13	21	10	9	8	64	48	12†

*Unique to other T_{cap} values. †Unique to other V_{pre} and V_{post} values. ‡Σ_{uniq} = 10 at T_{cap} = 190°C.

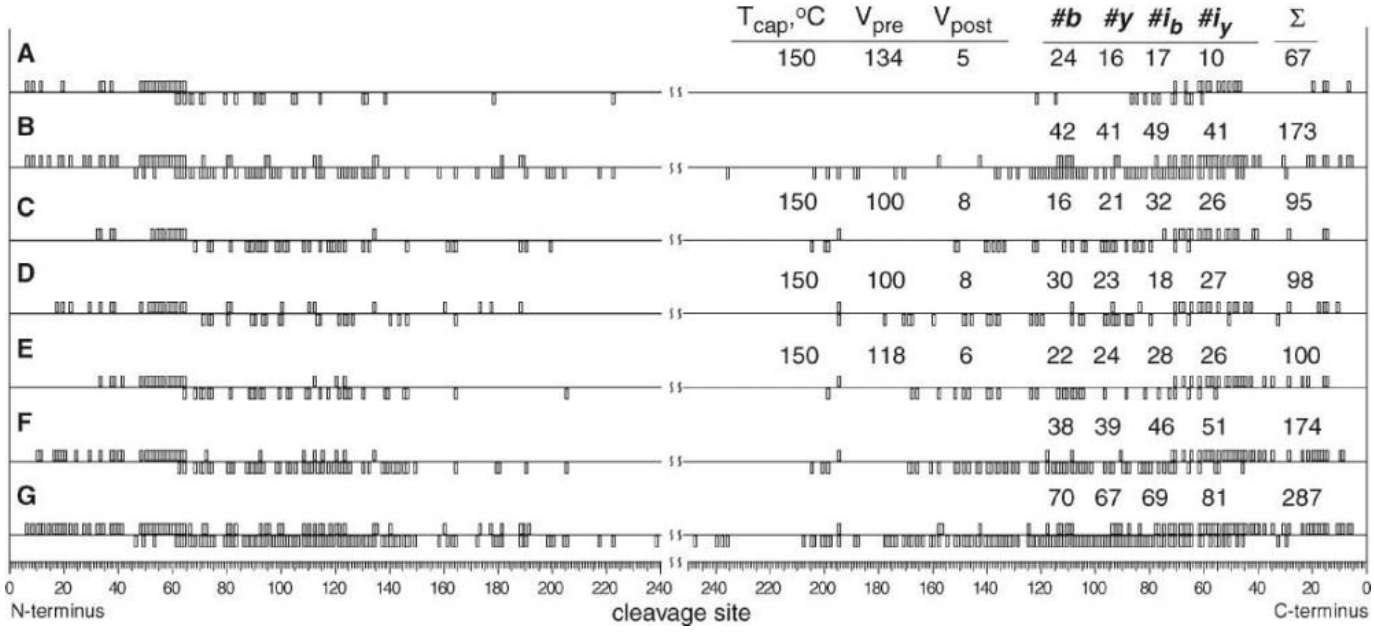


Fig. 3. PFD mass spectra of PurL (1314 residues): the effect of additives to the electrospray solution. (**A**) No additive. (**B**) Sum of 11 spectra without additives. Additives: (**C**) 500 μM ammonium citrate, (**D**) 500 μM ammonium succinate, and (**E**) 500 μM ammonium tartrate. (**F**) Sum of four spectra with 500 μM ammonium tartrate. (**G**) Sum of all 21 spectra. Designations as in Fig. 2.

its 27 Cys residues have not been specified (*24*). For such applications of bottom-up MS to distinguish S-H and S-S in glycosylated proteins (*25*), prior enzymatic or chemical treatment to remove glycans is necessary. By MS, Feng and Konishi measured a M_w of 196,863 ± 29 (*26*), indicating 5.3% glycosylation of the predicted amino acid sequence M_w of 186,437. Our ESI mass spectrum gave no M_w data, presumably because of molecular-ion heterogeneity due to partial deglycosylation. Gentle PFD (T_{cap} = 115°C, V_{pre} = 50, V_{post} = −4) gave, unexpectedly, two major fragment ions of 20,838 daltons (b_{185} of the β chain) and 165,746 ± 80 daltons, for a total of 186,584 ± 80 daltons, indicating that the original ~7% glycosylation had been reduced to <0.1% by PFD. Four spectra obtained with more strenuous activation (T_{cap} = 150° to 190°C, V_{pre} = 80 to 105 V, V_{post} = 10 to 14 V) identified 87 cleavages (Fig. 2, E to G) that yielded fragment ions without glycosylation.

These PFD data, simplified by the concomitant deglycosylation, can be used to assess possible disulfide bonding of the cysteine residues on the basis of two criteria. The presence of an S-S bond causes the experimental PFD mass to be 2 daltons less than the sequence-predicted value (*27*). Further, fragment ions are usually not observed from backbone cleavages between the Cys residues of an intrachain S-S bond, because this would require its additional dissociation. For the β chain with five cysteines (Fig. 2E), the masses of the $b_{<49}$ ions are those predicted, whereas the masses of the $b_{>78}$ ions are 2 daltons low, consistent with S-S bonding between the N-terminal Cys_{49} and Cys_{78} pair. The correspondingly low values for $y_{>41}$ ions indicate the C terminus S-S bond from Cys_{41} to Cys_7. The remaining β-chain Cys that is 109 residues from the C terminus must provide the interchain S-S bond; consistent with this prediction, many bonds are cleaved in the backbone between the two proposed intrachain S-S bonds.

For the central α chain (Fig. 2F), the b_{36} mass shows that Cys_{23} and Cys_{24} are unmodified, but the b_{56}, b_{60}, b_{86}, and b_{121} masses are 2 daltons less than expected, indicating S-S bond formation between two of the cysteines 37, 49, and 56. Similar evidence shows that the Cys that is 53 residues from the C terminus is unmodified, leaving the remaining cysteines 141, 197, and 442 as sites for previously identified S-S bonds to the outer β and γ chains (*24*). In the γ chain (Fig. 2G), eight fragment ions of $b_{\leq 69}$ have predicted mass values, indicating that Cys_{18} is unmodified. However, the 17 y ions of $y_{\leq 174}$ have mass values 2 daltons less than that predicted, indicating a C-terminal S-S bond between Cys_{18} and Cys_3 and –SH at the next seven cysteines. This result leaves only the N-terminal Cys_{82} and Cys_{113} as sites for the two interchain S-S bonds whose presence, but not locations, were found previously (*24*). These cysteines provide linkage to two of the proposed interchain S-S sites of the α chain, whose remaining site was linked to that of the β chain. For the fragment ion assignments shown in Fig. 2, E to G, the standard deviation of the mass difference in measured versus calculated values based on the S-S bonds postulated was ±0.14 dalton. Of the 27 cysteines in the C4 glycoprotein, eight are in previously unidentified S-S bonds, modifying the deglycosylated M_w value to 186,429 (*27*).

In initial studies, the linear protein mycocerosic acid synthase (Mas) from the construct pQLAB-Mas (*29*) gave a measured M_w of 228,934 ± 60, compared with the theoretical value of 229,067 (2154 residues). Five PFD spectra (T_{cap} = 150° to 190°C, V_{pre} = 70 to 125 V, V_{post} = 4 to 15 V) (fig. S1) gave 62 cleavages as far as 134 and 182 residues from the N and C termini, respectively, and the N-terminal cleavage data showed the absence of the predicted methionine (corrected theoretical M_w of 228,936 with 2153 residues).

For the identification of larger proteins by top-down MS (*5*), the limitation found here that primary b, y ions are formed by dissociations in only the first 200 amino acids of each terminus (~75% within 100 residues) has the advantage that the fragment ion mass values of the unknown sequences only have to be matched against masses of the correspondingly short terminal fragments of the DNA-predicted sequences, whereas the short peptides generated for bottom-up MS can be formed from any part of the protein. As a test of top-down identification of larger proteins (table S1), single PFD spectra of 1:1, 2:1, and 3:1 mixtures of PurL and β-Gal gave 11, 16, and 15, respectively, different mass values (1 to 10 kD, with an individual value often represented by multiple charge states) that matched those for primary backbone cleavages (b, y ions) predicted for PurL. These mixtures also gave 17, 13, and 11 b, y ions (the largest had 84 residues) that matched those for β-galactoside, and all mass values fit those predicted with a standard deviation of 4.9 parts per million. These results suggest that reliable identifications (*5*, *12*) for more complex mixtures of large proteins should be possible without initial proteolysis.

Top-down PFD characterization with our 6-T FTMS (12-T instruments now available have far higher resolving power and sensitivity) can provide even ~70% sequence coverage on the first ~200 residues of each terminus of a large protein. This study indicates the further applicability of PFD to characterize stable posttranslational modifications such as methylation, acetylation, oxidation, and deamidation (*27*) in large proteins.

References and Notes

1. N. L. Kelleher *et al.*, *J. Am. Chem. Soc.* **121**, 806 (1999).
2. J. B. Fenn, M. Mann, C. K. Meng, S. F. Wong, C. M. Whitehouse, *Science* **246**, 64 (1989).
3. K. D. Henry *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **86**, 9075 (1989).
4. F. W. McLafferty, E. K. Fridriksson, D. M. Horn, M. A. Lewis, R. A. Zubarev, *Science* **284**, 1289 (1999).
5. N. L. Kelleher, *Anal. Chem.* **76**, 197A (2004).
6. J. A. Loo, C. G. Edmonds, R. D. Smith, *Anal. Chem.* **63**, 2488 (1991).
7. N. L. Kelleher *et al.*, *Protein Sci.* **7**, 1796 (1998).
8. S. P. Gygi, R. Aebersold, *Curr. Opin. Chem. Biol.* **4**, 489 (2000).
9. W. H. McDonald, J. R. Yates, *Dis. Markers* **18**, 99 (2002).
10. W. J. Henzel, C. Watanabe, J. T. Stults, *J. Am. Soc. Mass Spectrom.* **14**, 931 (2003).
11. Y. Ge *et al.*, *J. Am. Chem. Soc.* **124**, 672 (2002).
12. D. M. Horn, R. A. Zubarev, F. W. McLafferty, *Proc. Natl. Acad. Sci. U.S.A.* **97**, 10313 (2000).
13. C. S. Hoaglund-Hyzer, A. E. Counterman, D. E. Clemmer, *Chem. Rev.* **99**, 3037 (1999).
14. K. Breuker, H.-B. Oh, D. M. Horn, B. A. Cerda, F. W. McLafferty, *J. Am. Chem. Soc.* **124**, 6407 (2002).
15. H. Oh *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **99**, 15863 (2002).
16. K. Breuker, F. W. McLafferty, *Angew. Chem. Int. Ed.* **44**, 4911 (2005).
17. H. Zhai, X. Han, K. Breuker, F. W. McLafferty, *Anal. Chem.* **77**, 5777 (2005).
18. J. A. Loo, C. G. Edmonds, R. D. Smith, *Science* **248**, 201 (1990).
19. Unless stated otherwise, proteins were from Sigma-Aldrich (St. Louis, MO) and desalted with a Michrom BioResources (Auburn, CA) protein trap. Protein solutions were ~5 μM in 50:45:5 MeCN:H_2O:AcOH; ~1 s of ESI is necessary to fill the FTMS cell for measurement of each scan, with ~7×10^{-13} mol consumed for a 50-scan spectrum. Apparatus (Finnigan 6T FTMS) and experimental details were described recently (*17*). There, temperatures of the inlet capillary were measured at its flared entrance end just outside the heating current connection; here temperatures were measured in the stainless-steel body, with comparative values of 56°/58°, 62°/90°, 76°/150°, and 100°/190°C. Also as justified there (*17*), the predicted sequence is used to assign the observed masses to the N- and C-terminal products (b and y ions, respectively) and to internal ions (i_b and i_y) from secondary b, y dissociation. Excluding minor ions from H_2O/NH_3 loss, this approach allowed >95% assignment of observed mass values, although obviously the b, y are more reliable than the i_b and i_y assignments. Singly charged fragment ions of low, but similar, relative intensities (~1%; up to 10% at T_{cap} = 190°C, V_{pre} = 100, V_{post} = 13) appear at nearly every mass from 600 to 1500 daltons, even with ESI solution additives. The FTMS resolving power allows THRASH (*20*) to recognize and subtract these ions (*17*).
20. D. M. Horn, R. A. Zubarev, F. W. McLafferty, *J. Am. Soc. Mass Spectrom.* **11**, 320 (2000).
21. R. H. Jacobson, X.-J. Zhang, R. F. DuBose, B. W. Matthews, *Nature* **369**, 761 (1994).
22. D. P. Little, J. P. Speir, M. W. Senko, P. B. O'Connor, F. W. McLafferty, *Anal. Chem.* **66**, 2809 (1994).
23. R. Anand, A. A. Hoskins, J. Stubbe, S. E. Ealick, *Biochemistry* **43**, 10328 (2004).
24. T. Seya, S. Nagasawa, J. Atkinson, *J. Immunol.* **136**, 4152 (1986).
25. J. Qin, B. T. Chait, *Anal. Chem.* **69**, 4002 (1997).
26. R. Feng, Y. Konishi, *Anal. Chem.* **64**, 2090 (1992).
27. A similar top-down approach successfully identified five deamidation (–NH_2 → –OH, +1 dalton) sites in ribonuclease A (13.7 kD) (*28*).
28. V. Zabrouskov *et al.*, *Biochemistry* **45**, 987 (2006).
29. K. C. Onwueme, C. J. Vos, J. Zurita, J. A. Ferreras, L. E. N. Quadri, *Prog. Lipid Res.* **44**, 259 (2005).

30. We thank S. Ealick and L. Quadri for purified PurL and Mas. We also thank them and B. Baird, T. Begley, H. Zhai, and G. Infusini for helpful discussions. This work was supported by the Institute of General Medical Sciences (NIH grant GM16609 to F.W.M.) and the Austrian Fonds zur Förderung der Wissenschaftlichen Forschung and Tiroler Wissenschaftsfonds (grant T229 and UNI-0404/158 to K.B.).

Supporting Online Material

www.sciencemag.org/cgi/content/full/314/5796/109/DC1
Fig. S1
Table S1

18 April 2006; accepted 16 August 2006
10.1126/science.1128868

Boryllithium: Isolation, Characterization, and Reactivity as a Boryl Anion

Yasutomo Segawa, Makoto Yamashita,* Kyoko Nozaki*

Nucleophilic, anionic boryl compounds are long-sought but elusive species. We report that reductive cleavage of the boron-bromine bond in *N*,*N*′-bis(2,6-diisopropylphenyl)-2-bromo-2,3-dihydro-1*H*-1,3,2-diazaborole by lithium naphthalenide afforded the corresponding boryllithium, which is isoelectronic with an *N*-heterocyclic carbene. The structure of the boryllithium determined by x-ray crystallography was consistent with sp^2 boron hybridization and revealed a boron-lithium bond length of 2.291 ± 0.006 angstroms. The structural similarity between this compound and the calculated free boryl anion suggests that the boron atom has an anionic charge. The ^{11}B nuclear magnetic resonance spectrum also supports the boryl anion character. Moreover, the compound behaves as an efficient base and nucleophile in its reactions with electrophiles such as water, methyl trifluoromethanesulfonate, 1-chlorobutane, and benzaldehyde.

For most of the *p*-block elements in the second row of the periodic table, anions can be prepared as lithium salts, such as lithium fluoride (LiF), lithium hydroxide (LiOH), lithium amide ($LiNH_2$), and methyllithium (H_3CLi) (*1*). However, there have been no direct observations of the lithium salt of anionic boron atom, boryllithium (a parent H_2BLi or its analog) (*2*). Since 1952, three examples (*3*–*5*) have been reported asserting the existence of alkali metal salts of anionic organoboron compounds as reactive intermediates, but none of these studies succeeded in spectroscopic characterization or isolation of the boryl anions (*6*–*16*). This lack of success presents a sharp contrast to the widely studied boron compounds containing a transition metal–boron bond (*17*, *18*) or a *p*-block main group element–boron bond (*2*).

As a target, we selected diamino-substituted boryllithium according to the theoretical prediction (*19*) that the diamino groups on the boron atom should stabilize boryllithium. The boron atom of boryllithium formally has six valence electrons, whereas the central atom meets the Lewis octet rule in the lithium salt of the other second-row *p*-block elements (Fig. 1A). Accordingly, attempts to abstract a proton from a B-H bond should result in the formation of a Lewis acid-base adduct (Fig. 1B, top) to satisfy the octet rule rather than deprotonation to form a boryl anion. Furthermore, electronegativity considerations predict lower stability for a boryl anion than for the corresponding carbanion (Pauling's electronegativity: C, 2.55; B, 2.04) (*20*). Thus, we selected the reduction of a diaminohaloborane (*5*) for our synthetic approach to boryllithium (Fig. 1B, bottom). We report the synthesis of boryllithium, its characterization in the solid state and in solution, and its reactions with several electrophiles.

Scheme 1 illustrates the synthesis of boryllithium. The B-Li bond of boryllithium can be drawn as a resonance between a covalent and an ionic bond. The isoelectronic relation of the boryl anion to a singlet carbene prompted us to synthesize boryllithium **3** as an isoelectronic analog of stable *N*-heterocyclic carbene **5** (Fig. 2) (*21*, *22*). Also, the parent boryl anion **6** was predicted to realize aromatic stabilization by the five-membered ring structure containing two nitrogen atoms (*23*, *24*). Large 2,6-diisopropylphenyl groups were introduced on the nitrogen atoms to prevent the boryl radical intermediates from dimerizing to diborane (*5*, *25*–*27*). The same combination of nitrogen-containing five-membered ring structure and 2,6-diisopropylphenyl groups has led to successful isolation of a free anionic gallium(I) species **7**, which is a heavier congener of boryl anion (*28*). A precursor *N*,*N*′-bis(2,6-diisopropylphenyl)-2-bromo-2,3-dihydro-1*H*-1,3,2-diazaborole (**2**) was synthesized in 56% yield through reduction of diimine **1** (*29*) by magnesium metal (*30*, *31*) followed by reaction with BBr_3. The bromoborane **2** was successfully reduced to the boryllithium **3** with the use of a combination of lithium powder and naphthalene in tetrahydrofuran (THF) at −45°C. The reaction of **3** with water quantitatively gave a hydroborane **4**, which could be independently synthesized by reaction of **2** with $LiAlH_4$ (*32*). Treatment of **3** with D_2O afforded the corresponding deuterioborane **4-D** in 82% yield, which indicates that **4** was mostly generated from the reaction of **3** with water rather than with the solvents or ligand backbone.

The use of 1,2-dimethoxyethane (DME) as a reaction solvent in place of THF enabled crystallization of boryllithium **3** with an included DME molecule. The structure of boryllithium **3**–DME was confirmed by x-ray crystallographic analysis with thermally unstable single crystals obtained from a hexane solution of **3**-DME at −45°C (Fig. 3). The solid-state structure of **3**-DME reveals a B-Li bond of 2.291 ± 0.006 Å, which is 8.5% longer than the sum of the covalent radii (2.11 Å) of boron and lithium atoms (*33*). A cocrystallized DME coordinates to the lithium atom, and one of the two DME oxygen atoms bridges to the other lithium atom to form a dimeric structure.

Structural comparison of **3**-DME with related compounds (Table 1) shows that the two B-N bond lengths (1.465 ± 0.004 Å and 1.467 ± 0.004 Å) in **3**-DME are longer than those (1.418 ± 0.003 Å and 1.423 ± 0.003 Å) in the hydroborane **4**, and the angle of N1-B1-N2 (99.2° ± 0.2°) in **3**-DME is smaller than that in **4** (105.25° ± 0.16°). These B-N lengths and the

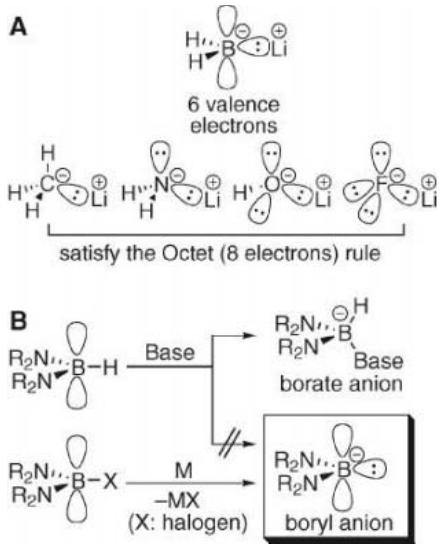
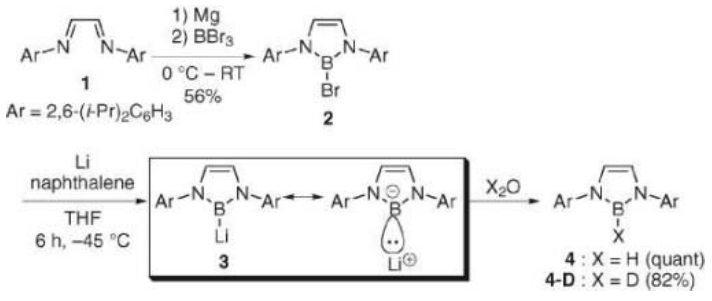


Fig. 1. (**A**) Parent lithium salts of second-row *p*-block elements except argon. (**B**) Two conceivable pathways to synthesize the boryl anion.



Scheme 1.

Department of Chemistry and Biotechnology, Graduate School of Engineering, University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, 113-8656, Japan.

*To whom correspondence should be addressed. E-mail: makotoy@chembio.t.u-tokyo.ac.jp (M.Y.); nozaki@chembio.t.u-tokyo.ac.jp (K.N.)

N-B-N angle in **3**-DME are closer to those in the previously calculated free boryl anion **6** (B-N = 1.475 Å; N-B-N = 97.72°) (*23*) than to those in **4**, consistent with a highly polarized B-Li bond and anionic character of the boron center in **3**. The structural change from **4** to **3**-DME is similar to that from the corresponding imidazolium salt **8** to singlet carbene **5** (*34*). Although the central dihydrodiazaborole ring in **3**-DME is planar, the lithium atom is slightly above (0.2481 Å) the mean plane of the dihydrodiazaborole ring. The N1-B1-Li1 (136.1° ± 0.2°) and N2-B1-Li1 (123.9° ± 0.2°) angles apparently differ, although the sum of the angles (359.2°) around the central boron atom shows a planarity characteristic of an sp^2-hybridized boron atom. These local distortions from ideal sp^2 hybridization of the central boron atom, without significant distortion of the whole dihydrodiazaborole ring, may reflect an ionic character of the B-Li bond.

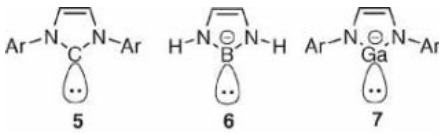


Fig. 2. Reference compounds [Ar = 2,6-(*i*-Pr)$_2$C$_6$H$_3$].

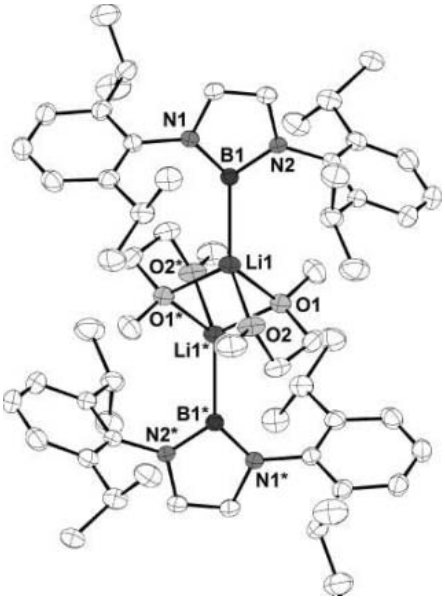
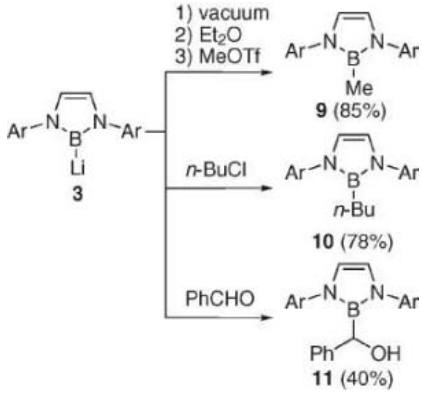


Fig. 3. Crystal structure of **3**-DME (hydrogen atoms were omitted for clarity; half of the whole structure constitutes an asymmetric unit where numbers with asterisks are in the second asymmetric unit; only the major portions of disordered isopropyl groups are illustrated). Selected bond distances and angles: B1-Li1 = 2.291 ± 0.006 Å, B1-N1 = 1.467 ± 0.004 Å, B1-N2 = 1.465 ± 0.004 Å, N1-B1-N2 = 99.2° ± 0.2°, N1-B1-Li1 = 136.1° ± 0.2°, N2-B1-Li1 = 123.9° ± 0.2°.

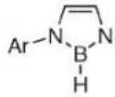
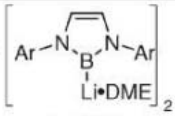
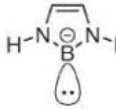
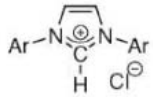
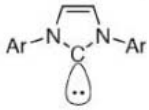
The boryllithium compound **3**-DME was also spectroscopically characterized in THF-d_8 solution, although 7% of **3** decomposed to the corresponding hydroborane **4** during sample preparation. In the ^{1}H nuclear magnetic resonance (NMR) spectrum of **3**-DME in THF-d_8, two distinct methyl resonances were observed, reflecting inhibited rotation around the Ar-N bond likely due to steric repulsion. In contrast, the two 2,6-diisopropylphenyl rings were equivalent in the spectrum, indicating that there are two mirror planes in **3**, one coplanar to the diazaborole ring and the other perpendicular to it. The DME molecule in **3**-DME was found to dissociate from the lithium atom in solution (free DME resonances were observed at 3.42 and 3.26 ppm). Instead, THF molecules appeared to coordinate to the lithium atom in THF-d_8 solution; preparation of **3** from **2** in THF-d_8 produced identical NMR spectra. The ^{11}B NMR spectrum of **3** showed a resonance at 45.4 ppm with a large half-width of $h_{1/2}$ = 535 Hz. The lower field shift and larger half-width of this peak relative to those of hydroborane **4** (δ_B 22.9 ppm, $h_{1/2}$ = 379 Hz) can be attributed to the paramagnetic contribution to nuclear shielding by the low-lying transition from an sp^2 lone pair of the boryl anion to the π* orbital on the dihydrodiazaborole ring, as was reported for the isoelectronic singlet diaminocarbene (*35*). Absence of splitting in the proton-coupled ^{11}B NMR spectrum of **3** confirmed that there is no B-H bond in **3**, whereas a ^{11}B-^{1}H coupling constant of 154 Hz was detected for **4**. The ^{7}Li NMR spectrum of **3** showed a broad singlet peak at 0.46 ppm with a half-width of $h_{1/2}$ = 36 Hz; the large half-width may originate from the interaction of the lithium with the quadruple boron nucleus (*36*). Thus, the NMR spectroscopy of boryllithium **3** also indicates that the central boron atom has an anionic character with a highly polarized B-Li bond in solution.



Scheme 2.

After removing excess lithium from the THF solution, boryllithium **3** was shown to react with a variety of electrophiles (Scheme 2). In ether, compound **3** reacted with methyl trifluoromethanesulfonate to give the substituted product methylborane **9** in 85% yield. The reaction of **3** with 1-chlorobutane in THF gave *n*-butylborane **10** in 78% yield. Boryllithium **3** also attacked the carbonyl group of benzaldehyde to afford the corresponding adduct, α-borylbenzyl alcohol **11** in 40% yield (*37*). The x-ray structure of **11** (see supporting online material) revealed that the hydroxy group does not interact (intra- or intermolecularly) with the central boron atom to form three-membered (*38*) or six-membered (*39*, *40*) rings and that there is no intermolecular hydrogen bonding. Thus, we have successfully demonstrated that boryllithium **3** behaves as a base or a nucleophile. Historically, the area of organic chemistry has been widely expanded by discoveries of new chemical reagents. We believe the present boryllithium opens important synthetic pathways to boron-containing compounds.

Table 1. Structural comparison of boryllithium **3** with related compounds [Ar = 2,6-(*i*-Pr)$_2$C$_6$H$_3$].

compounds					
	4	**3**-DME	**6**	**8**	**5**
B(or C)-N (Å)	1.418(3) 1.423(3)	1.465(4) 1.467(4)	1.475	1.341 1.338	1.364 1.369
N-B(or C)-N (°)	105.25(16)	99.2(2)	97.72	107.6	101.4
reference	this work	this work	23	34	34

References and Notes

1. A. R. Katritzky, O. Meth-Cohn, C. W. Rees, Eds., *Organolithium Methods* (Academic Press, London, 1988).
2. H. Nöth, in *Product Subclass 4: Metalloboranes*, D. E. Kaufmann, Ed. (Thieme, Stuttgart, 2005), pp. 139–178.
3. R. W. Auten, C. A. Kraus, *J. Am. Chem. Soc.* **74**, 3398 (1952).
4. J. L. R. Williams *et al.*, *J. Am. Chem. Soc.* **89**, 5153 (1967).
5. L. Weber, M. Schnieder, P. Lonnecke, *J. Chem. Soc. Dalton Trans.* **2001**, 3459 (2001).
6. R. Köster, G. Benedikt, *Angew. Chem. Int. Ed. Engl.* **2**, 219 (1963).
7. R. Köster, G. Benedikt, *Angew. Chem. Int. Ed. Engl.* **3**, 515 (1964).
8. K. Smith, K. Swaminathan, *J. Chem. Soc. Dalton Trans.* **1976**, 2297 (1976).
9. J. D. Wilkey, G. B. Schuster, *J. Org. Chem.* **52**, 2117 (1987).
10. S. Boyatzis, J. D. Wilkey, G. B. Schuster, *J. Org. Chem.* **55**, 4537 (1990).
11. As examples of sp^3 boron anions, the existence of base-stabilized dihydroboryl anions (base-$BH_2^-M^+$, where base is amine or phosphine) were suggested as intermediates in reactions with electrophiles. See (*12*–*15*). Also, bishomotriboriranide may be regarded as a boryl anion equivalent where the boron anion is electronically stabilized

by a neighboring diboron unit to form an sp-hybridized boron atom. However, there is no B-Li bond in this molecule. See (*16*).
12. T. D. Parsons, J. M. Self, L. H. Schaad, *J. Am. Chem. Soc.* **89**, 3446 (1967).
13. B. R. Gragg, G. E. Ryschkewitsch, *Inorg. Chem.* **15**, 1209 (1976).
14. A. Blumenthal, P. Bissinger, H. Schmidbaur, *J. Organomet. Chem.* **462**, 107 (1993).
15. T. Imamoto, T. Hikosaka, *J. Org. Chem.* **59**, 6753 (1994).
16. M. Unverzagt *et al.*, *Angew. Chem. Int. Ed. Engl.* **36**, 1469 (1997).
17. H. Braunschweig, M. Colling, *Coord. Chem. Rev.* **223**, 1 (2001).
18. G. J. Irvine *et al.*, *Chem. Rev.* **98**, 2685 (1998).
19. M. Wagner, N. J. R. van Eikema Hommes, H. Nöth, P. v. R. Schleyer, *Inorg. Chem.* **34**, 607 (1995).
20. K. E. Laidig, A. Streitwieser, *J. Comput. Chem.* **17**, 1771 (1996).
21. A. J. Arduengo III, R. L. Harlow, M. Kline, *J. Am. Chem. Soc.* **113**, 361 (1991).
22. H.-W. Wanzlick, *Angew. Chem. Int. Ed. Engl.* **1**, 75 (1962).
23. N. Metzler-Nolte, *New J. Chem.* **22**, 793 (1998).
24. A. Sundermann, M. Reiher, W. W. Schoeller, *Eur. J. Inorg. Chem.* **1998**, 305 (1998).
25. R. J. Brotherton, A. L. McCloskey, L. L. Petterson, H. Steinberg, *J. Am. Chem. Soc.* **82**, 6242 (1960).
26. V. M. Dembitsky, H. Abu Ali, M. Srebnik, *Adv. Organomet. Chem.* **51**, 193 (2004).
27. T. B. Marder, in *Product Subclass 3: Diborane(4) Compounds*, D. E. Kaufmann, Ed. (Thieme, Stuttgart, 2005), pp. 117–137.
28. E. S. Schmidt, A. Jockisch, H. Schmidbaur, *J. Am. Chem. Soc.* **121**, 9758 (1999).
29. M. B. Abrams, B. L. Scott, R. T. Baker, *Organometallics* **19**, 4944 (2000).
30. L. Weber *et al.*, *Chem. Ber.* **130**, 705 (1997).
31. I. L. Fedushkin *et al.*, *Eur. J. Inorg. Chem.* **2003**, 3336 (2003).
32. L. Weber *et al.*, *Eur. J. Inorg. Chem.* **1999**, 491 (1999).
33. J. Emsley, *The Elements* (Oxford Univ. Press, New York, ed. 3, 1998), pp. 40, 52.
34. A. J. Arduengo III, R. Krafczyk, R. Schmutzler, *Tetrahedron* **55**, 14523 (1999).
35. A. J. Arduengo *et al.*, *J. Am. Chem. Soc.* **116**, 6361 (1994).
36. N. P. Rath, T. P. Fehlner, *J. Am. Chem. Soc.* **110**, 5345 (1988).
37. The related compounds, Cy_3P-BH_2-CH(OH)Ph, have been reported by the reaction of Cy_3P-BH_2Li with PhCHO in (*15*).
38. Diaminoboryllithium [$(H_2N)_2$BLi] was calculated to react with formaldehyde to form a three-membered ring structure consisting of B, C, and O atoms. See (*20*).
39. Acylborane [R_2BC(=O)R′] was postulated to dimerize to form a six-membered ring structure consisting of B, C, and O atoms. See (*40*).
40. S. Günter, H. Nöth, *Chem. Ber.* **101**, 2502 (1968).
41. We thank T. Kawashima and K. Goto for the use of an x-ray diffractometer for **4**, and N. Tokitoh and T. Sasamori for data processing of **3**-DME. Supported by Grant-in-Aid for Scientific Research on Priority Areas 17065005 (Advanced Molecular Transformations of Carbon Resources) and for Young Scientists (B 18750027) from Ministry of Education, Culture, Sports, Science and Technology, Japan, and by a Takeda Pharmaceutical Company Award in Synthetic Organic Chemistry. Structural parameters for compounds **3**-DME, **4**, and **11** are available free of charge from the Cambridge Crystallographic Data Centre under numbers CCDC-604926, 611434, and 604927, respectively.

Supporting Online Material

www.sciencemag.org/cgi/content/full/314/5796/113/DC1
Materials and Methods
Figs. S1 and S2
Table S1

29 June 2006; accepted 25 August 2006
10.1126/science.1131914

Unraveling the Mystery of Indian Monsoon Failure During El Niño

K. Krishna Kumar,[1] Balaji Rajagopalan,[2,3] Martin Hoerling,[4]* Gary Bates,[4] Mark Cane[5]

The 132-year historical rainfall record reveals that severe droughts in India have always been accompanied by El Niño events. Yet El Niño events have not always produced severe droughts. We show that El Niño events with the warmest sea surface temperature (SST) anomalies in the central equatorial Pacific are more effective in focusing drought-producing subsidence over India than events with the warmest SSTs in the eastern equatorial Pacific. The physical basis for such different impacts is established using atmospheric general circulation model experiments forced with idealized tropical Pacific warmings. These findings have important implications for Indian monsoon forecasting.

Climate is the decisive influence on habitation and subsistence of India's burgeoning population. India's wealth is measured by its agricultural output, and now even modest harvest failures result in exaggerated economic and societal consequences. Swings in crop abundances are propelled by the year-to-year successes of the summer (June to September) monsoon rains (*1*). As a result, monsoon predictions are achieving new importance for setting into motion timely and effective preparedness and mitigation activities. The predictions themselves can be as influential as the actual verified monsoon rainfall, as happened for Zimbabwe during 1997 when drought predictions led to curtailment of bank loans for agricultural development (*2*). A similar situation, also during 1997, occurred in India when a much-touted prediction of poor monsoon rains proved false. A more painful scenario unfolded during 2002 and 2004 (*3*, *4*) when normal monsoon rains were predicted but severe drought materialized for which no contingencies were in place.

In many seasonal forecast tools, Indian monsoon rains are predicted to vary in direct proportion to the strength of the El Niño Southern Oscillation (ENSO) phenomenon in the tropical Pacific (*5*–*7*), measured, for example, by the standardized NINO3 index (*8*). Indeed, years with moderate to extreme cold states (NINO3 index < -1), have had abundant monsoon rains without exception. On the other hand, years of moderate to extreme warm states have not been reliably dry. As seen in Fig. 1, the six leading droughts (*8*) since 1871 have occurred in tandem with a standardized NINO3 index exceeding $+1$, but the presence of El Niños has not guaranteed drought. No simple association describes the relation between the Indian monsoon and NINO3 SSTs when moderate to strong El Niño conditions exist; almost a full range of monsoon rains have accompanied SST warmings. For example, 1997 was the century's strongest El Niño, although no drought occurred, whereas the moderate El Niño of 2002

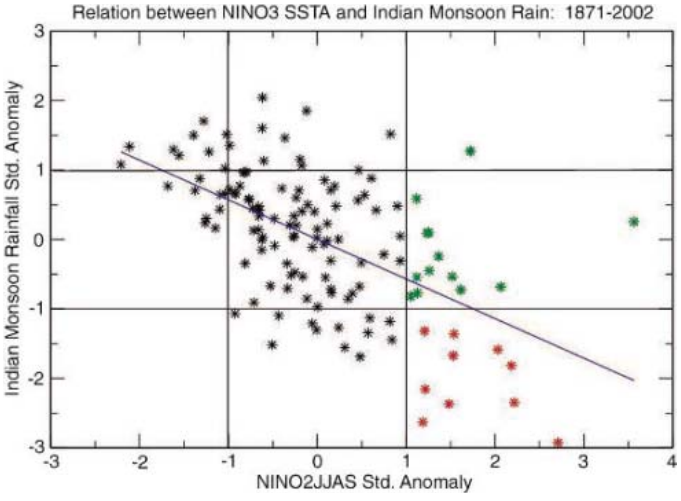


Fig. 1. Plot of standardized, all-India summer [June to September (JJAS)] monsoon rainfall and summer NINO3 anomaly index. Severe drought and drought-free years during El Niño events (standardized NINO3 anomalies > 1) are shown in red and green, respectively.

[1]Indian Institute of Tropical Meteorology, Pune, 411008, India. [2]Department of Civil Environmental and Architectural Engineering, University of Colorado, Boulder, CO 80309, USA. [3]Cooperative Institute for Research in Environmental Sciences, University of Colorado, Boulder, CO 80309, USA. [4]National Oceanic and Atmospheric Administration, Earth System Research Laboratory, Boulder, CO 80305, USA. [5]Lamont-Doherty Earth Observatory, Columbia University, Palisades, NY 10964, USA.

*To whom correspondence should be addressed. E-mail: martin.hoerling@noaa.gov

was accompanied by one of the worst Indian droughts of the past century (*4*). Such ambiguity undermines the utility of monsoon predictions for mitigation of drought's societal impacts.

Two hypotheses have been proposed to explain this ambiguity in the El Niño–Indian monsoon relationship. One is that chaotic variability in rainfall on intraseasonal time scales masks the remote effect of El Niño. Accordingly, the failure (abundance) of monsoon rains during 2002 (1997) would be viewed as the accidental behavior of an inherently noisy monsoon system, and the poor forecasts for these particular cases were the consequence of an only marginally predictable system. The other is that the Indian monsoon is highly sensitive to the details of tropical east Pacific sea surface warming. It is widely believed that El Niño's impact on the Indian monsoon is through the east-west displacement of the ascending and descending branches of the Walker circulation that link Indo-Pacific climates (*9*, *10*). Unusually warm waters during El Niño cause an increased ascent associated with increased rainfall. Mass continuity requires increased descent broadly over southeast Asia, suppressing monsoon rains. The hypothesis we explore is that the strength and position of these branches vary coherently with the details of El Niño warming.

We begin by examining the 23 strong El Niño years for atmosphere and ocean conditions that distinguish the 10 Indian monsoon droughts (red asterisks in Fig. 1) from the 13 drought-free years (green asterisks in Fig. 1). Figure 2A illustrates their contrasting sea surface temperatures (SSTs). The most notable difference in the tropical Pacific SSTs is the greater central Pacific warming during failed Indian monsoon years (Fig. 2A). These analyses suggest that India is more prone to drought when the ocean-warming signature of El Niño extends westward. Figure 2B displays the difference in tropical rainfall for the drought versus drought-free El Niño years. Although rainfall data are based on a smaller sample of cases for which satellite rainfall estimates are available, a physical consistency with the underlying SST anomalies in Fig. 2A is apparent. Increased rainfall occurs over the enhanced warmth of central Pacific Ocean waters, and the satellite estimates confirm dryness over India, the Indian Ocean, and other portions of Southeast Asia, indicating a wide reach to the drought signal. These rainfall anomalies form a dynamical couple that is linked by an Indo-Pacific anomalous Walker circulation, as seen in the velocity potential (*8*) at 200 hPa (Fig. 2B, contours).

The composite anomaly differences highlighted by shading in Fig. 2, A and B, are statistically significant (*8*) and are physically consistent with the expected rainfall-SST relationship. This is further seen by the separability of the probability density functions (PDFs) (*8*) of rainfall for drought versus drought-free years (Fig. 2C). Although this empirical analysis does not establish causal linkages, it does suggest that the two "flavors" of El Niño (*11*) result in significantly different responses in the Indian monsoon. The SST patterns of these two flavors can be described by a linear combination of the two leading, preferred patterns of tropical Pacific SST variability of the past half century (*8*), shown in Fig. 3. The first leading pattern (Fig. 3A) represents the overall strength of the ENSO events, and its associated temporal pattern is highly correlated with fluctuations in the NINO3 index (Fig. 3C). The second pattern (Fig. 3B) has polarity of opposite sign between

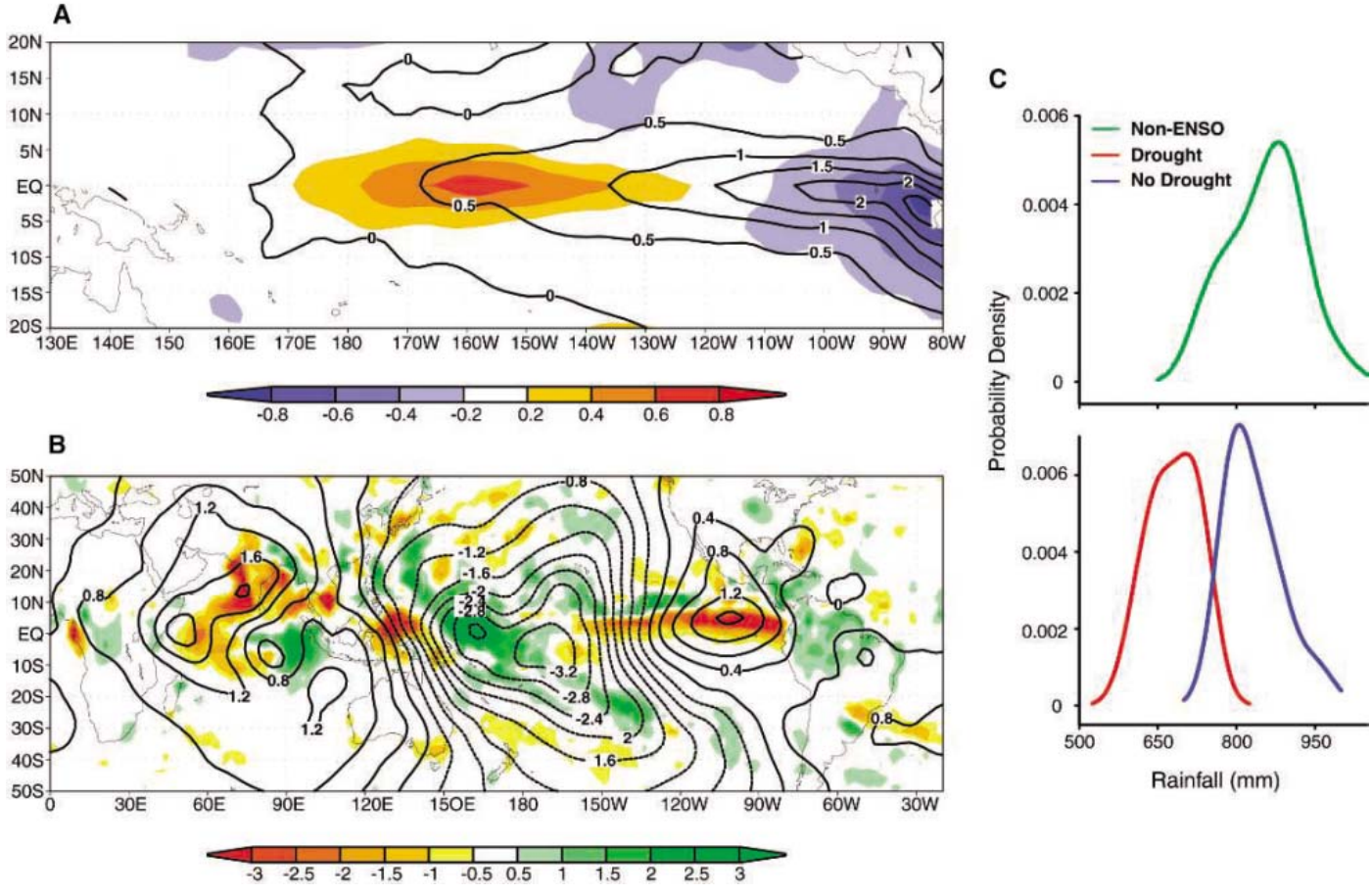


Fig. 2. (**A**) Composite SST difference pattern between severe drought (shaded) and drought-free El Niño years. Composite SST anomaly patterns of drought-free years are shown as contours. (**B**) Composite difference pattern between severe drought and drought-free years of velocity potential (contours) and rainfall (shaded). (**C**) PDF of all-India summer monsoon rainfall from severe-drought (red curve) and drought-free (blue curve) years associated with El Nino occurrence and from the non-ENSO years (green curve). SST and velocity potential composite differences are based on 1950 to 2004, rainfall composites are based on 1979 to 2004, and PDFs are based on 1873 to 2004.

the tropical Central and Eastern Pacific, and its temporal pattern is highly correlated with fluctuations of an index that measures the SST gradient across the Pacific basin (*8*) (Fig. 3D). We note in particular that the second leading pattern closely resembles the SST difference between severe drought and drought-free monsoon years (Fig. 2A, shaded).

General circulation model (GCM) experiments (*8*), forced with SST patterns resulting from linear combinations of the first two leading patterns of tropical Pacific SST variability, are used to test the hypothesis that "westward-shifted" Pacific Ocean warm events drive more intense sinking over the Indian region, initiating severe drought. Using National Center for Atmospheric Research–Community Climate Model Version 3, we performed four ensemble sets of experiments: (i) a 150-year control run of the GCM forced by monthly evolving global climatological mean SST; (ii) a fixed SST pattern resulting from the addition of the first two leading tropical Pacific SST patterns superimposed on the monthly evolving climatological SSTs globally; (iii) same as (ii), but subtracting the second leading tropical Pacific SST pattern from the first; and (iv) an SST pattern corresponding to the first leading pattern (i.e., Fig. 3A) alone. The model experiments for (ii), (iii), and (iv) were performed for a range of imposed SST warmth from 0 to +3 standard deviations (SD), with results available at an interval of 0.2 SD. We analyzed 10 simulations with different initial atmospheric conditions for each of these incremental warmings. Climatological SSTs were prescribed outside the tropical Pacific in these experiments.

Figure 4 illustrates two key aspects of the SST forcing of our atmospheric general circulation model that mimic the empirically derived patterns of Fig. 2. Contours in Fig. 4A are analogous to the amplitude and structure of the composite El Niño SSTs for drought-free years and correspond to the +2 SD experiment described above (iii). Shading in Fig. 4A is analogous to the observed SST structure that discriminates severe drought from drought-free El Niño years and corresponds to the difference between the +2 SD SST forcings of experiments (ii) and (iii). The ensemble mean rainfall and 200 hPa velocity potential difference between experiments (ii) and (iii) are shown in Fig. 4B. Notice a large-scale enhanced drought over the Indian region consistent with enhanced subsidence, and vice versa, in the tropical central Pacific. The similarity of this figure to that noted from the observations (Fig. 2B) is striking. This supports the hypothesis that it is the westward-shifted El Niño events that weaken the Indian monsoon severely.

The behavior of Indian monsoon rainfall under climatological SST conditions (control) and also under the anomalous conditions described by the three different SST patterns of experiments (ii, iii, and iv) is assessed through the construction of PDFs (*8*) that sample all the simulations of Indian rainfall drawn from the separate enactments of the GCM experiments (Fig. 4C).

The PDFs of the experiments using moderate SST warming are not separated, and mean Indian rainfall is only slightly less than the control experiment. For the stronger warming, however, the PDFs of the simulated rainfall are well separated and the median rainfall values are far below those of the control experiment. Under the influence of stronger SST forcing, the PDFs of the experiments that correspond to summing and differencing the two leading tropical Pacific SST patterns fall on the dry and the wet side, respectively, of the median rainfall from the experiment using only the leading SST pattern. This indicates that the leading SST pattern in itself produces droughts in India when of sufficient amplitude, but depending on the sign of the superposed second leading SST pattern, the droughts in India are either strong or weak, with a clear separation in the PDFs.

Identical experiments using two other climate models yield similar results (fig. S1). Also, experiments using the actual SST difference of Fig. 2A (*8*) reproduce the results based on using the idealized SST forcings (fig. S2). For all SST forcings, the PDFs of monsoon rainfall (Fig. 4C) are not sharply peaked but involve a considerable range of possible outcomes. The rainfall spreads are not materially different for the unforced control experiments compared with the strongly forced runs. This illustrates the influence of omnipresent internal atmospheric variability, although such in situ variability alone appears to be insufficient to generate severe monsoon failure.

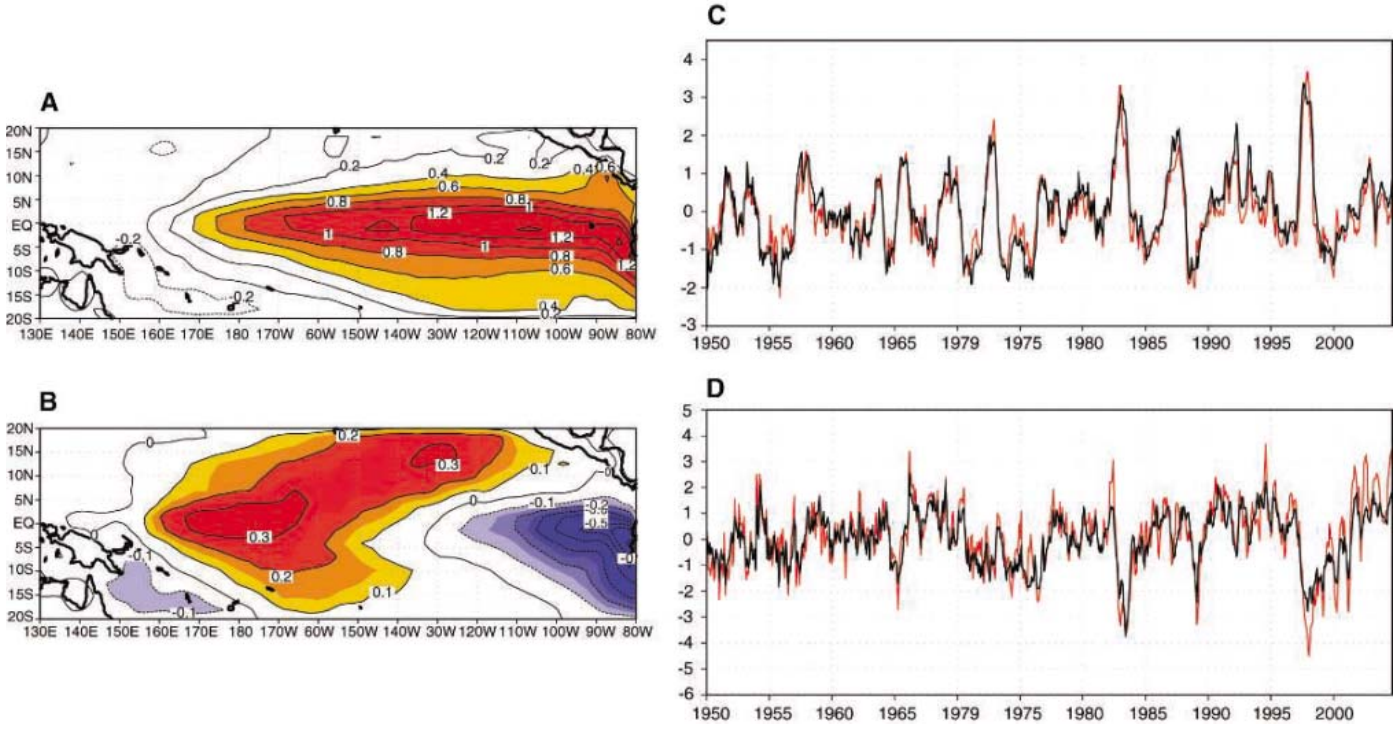


Fig. 3. (**A**) The first leading pattern of the tropical Pacific SST variability. (**B**) Same as (A) but for the second leading pattern. (**C**) The first leading temporal pattern (black line) overlaid with the monthly NINO3 index (red line). (**D**) The second leading temporal pattern (black line) overlaid with the trans-Niño index (TNI) (red line).

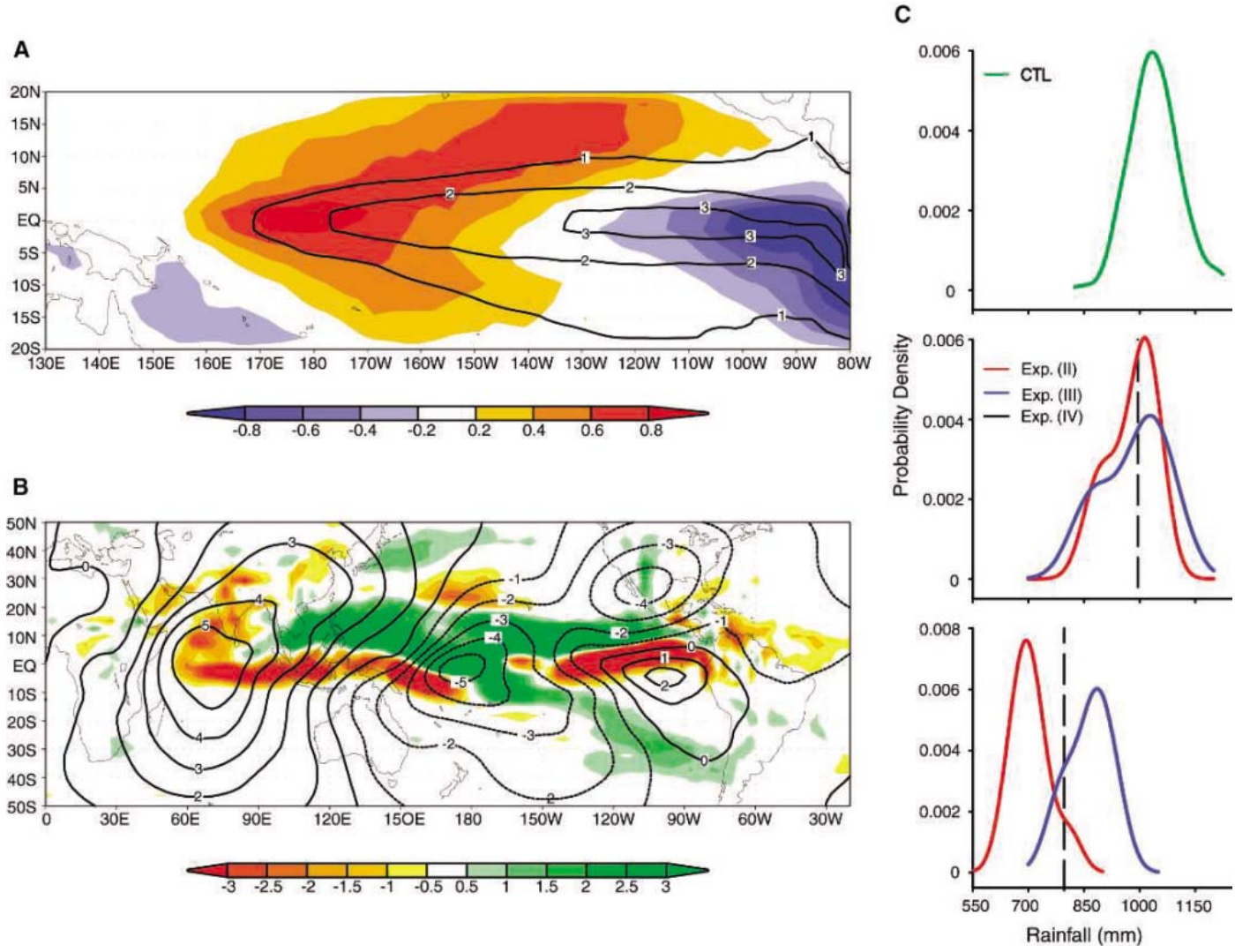


Fig. 4. (**A**) Contours are of the +2 SD experiment (iii) and are analogous to the amplitude and structure of the composite El Niño SSTs for drought-free years in Fig. 2A. Shadings are of the difference between the +2 SD SST forcings of experiments (ii) and (iii) and are analogous to the observed SST structure that discriminates severe drought from drought-free El Niño years. (**B**) The ensemble mean rainfall (shading) and 200 hPa velocity potential (contour) differences between experiments (ii) and (iii). (**C**) The PDF of the Indian monsoon rainfall corresponding to (top) the control, or unforced, experiment (i) green curve; and (middle and bottom) the forced experiments (ii) red curve, (iii) blue curve, and (iv) dashed line with +1 SD (middle) and +2 SD (bottom) imposed SST anomalies. For forced experiment (iv), only the median value (dashed line) is shown. For the forced experiments, each PDF is estimated from 30 ensembles. The model rainfall has been averaged over the Indian monsoon region of 8° to 30°N, 70° to 90°E.

The simulations demonstrate a strong dependence of the Indian monsoon on the tropical Pacific SST anomaly pattern associated with different El Niños. These results do not rule out an independent role for the Indian Ocean, as suggested in other studies (*12*–*14*), or in combination with ENSO (*15*). Regarding the mechanism by which the two flavors of El Niño yield different monsoon impacts, our study has suggested only one candidate, namely, a sensitivity of the tropical Walker circulation. The role of other plausible mechanisms, such as El Niño–monsoon teleconnections from the extratropics (*16*), or the role of reorganized weather disturbances, require further investigation.

The fact that the spatial configuration of tropical Pacific SST anomalies has a significant impact on the Indian monsoon indicates that traditional monsoon forecast methods using predictors that essentially capture the ENSO's strength are likely to be unsuccessful (*17*) in years when the spatial configuration of the SST anomalies is inconsistent with its strength; 1997, 2002, and 2004 are some of the recent years that attest to this. Incorporation of SST configuration information in the statistical models should improve monsoon forecasting skill (fig. S3). There is also the intriguing question of whether either of these flavors of tropical Pacific warmings will become preferred as a consequence of the ocean's response to human-induced changes in Earth's atmosphere's chemical composition. Whereas the consensus of climate change models points to an El Niño–like warming pattern of the tropical Pacific (*18*, *19*), the results of this study indicate that details of that human-induced ocean warming could have material consequences for the monsoon intensity over India.

References and Notes

1. K. Krishna Kumar, K. Rupa Kumar, R. G. Ashrit, N. R. Deshpande, J. W. Hansen, *Int. J. Climatol.* **24**, 1375 (2004).
2. S. G. Philander, *Our Affair with El Niño* (Princeton Univ. Press, Princeton, NJ, 2004).
3. S. Gadgil *et al.*, *Curr. Sci.* **83**, 394 (2002).
4. S. Gadgil, M. Rajeevan, R. Nanjundiah, *Curr. Sci.* **88**, 1389 (2005).
5. S. Hastenrath, *Climate Dynamics of the Tropics* (Kluwer Academic Publishers, Dordrecht, Netherlands, 1991).
6. K. Krishna Kumar, M. K. Soman, K. Rupa Kumar, *Weather* **50**, 449 (1995).
7. J. Shukla, D. A. Paolino, *Mon. Weather Rev.* **111**, 1830 (1983).
8. Materials and methods are available as supporting material on *Science* Online.
9. J. Shukla, in *Monsoons*, J. S. Fein, P. L. Stephens, Eds. (Wiley, New York, 1987).
10. C. F. Ropelewski, M. S. Halpert, *Mon. Weather Rev.* **115**, 1606 (1987).
11. K. Trenberth, *Proc. 18th Ann. Clim. Diagnost. Workshop*, Boulder, CO, **50** (1993).
12. N. H. Saji, B. N. Goswamy, P. N. Vinayachandran, T. Yamagata, *Nature* **401**, 360 (1999).

13. S. Gadgil, P. N. Vinayachandran, P. A. Francis, S. Gadgil, *Geophys. Res. Lett.* **31**, L12213 (2004).
14. K. Krishna Kumar, M. Hoerling, B. Rajagopalan, *Geophys. Res. Lett.* **32**, L08704 (2005).
15. H. Annamalai, P. Liu, *Q. J. R. Meteorol. Soc.* **131**, 805 (2005).
16. B. N. Goswami, P. K. Xavier, *Geophys. Res. Lett.* **32**, L8717, doi: 10.1029/2005GL023216 (2005).
17. K. Krishna Kumar, B. Rajagopalan, M. Cane, *Science* **284**, 2156 (1999).
18. M. A. Cane *et al.*, *Science* **275**, 957 (1997).
19. Intergovernmental Panel on Climate Change, *Climate Change 2001: The Scientific Basis: Summary for Policy Makers and Technical Summary of the Working Group 1 Report* (Cambridge Univ. Press, Cambridge, 2001).
20. We acknowledge funding support from Cooperative Institute for Research in Environmental Sciences and Asia Pacific Network to K.K. and from NOAA's Office of Global Programs to M.H. We also thank T. Xu and J. Eischeid for their contributions.

Supporting Online Material
www.sciencemag.org/cgi/content/full/1131152/DC1
Materials and Methods
Figs. S1 to S3

12 June 2006; accepted 29 August 2006
Published online 7 September 2006;
10.1126/science.1131152
Include this information when citing this paper.

Large Punctuational Contribution of Speciation to Evolutionary Divergence at the Molecular Level

Mark Pagel,* Chris Venditti, Andrew Meade

A long-standing debate in evolutionary biology concerns whether species diverge gradually through time or by punctuational episodes at the time of speciation. We found that approximately 22% of substitutional changes at the DNA level can be attributed to punctuational evolution, and the remainder accumulates from background gradual divergence. Punctuational effects occur at more than twice the rate in plants and fungi than in animals, but the proportion of total divergence attributable to punctuational change does not vary among these groups. Punctuational changes cause departures from a clock-like tempo of evolution, suggesting that they should be accounted for in deriving dates from phylogenies. Punctuational episodes of evolution may play a larger role in promoting evolutionary divergence than has previously been appreciated.

The theory of punctuated equilibrium as a description of evolution suggests that evolutionary divergence among species is characterized by long periods of stability or stasis followed by short punctuational bursts of evolution associated with speciation. Despite years of work on punctuational change, the theory remains contentious (*1*–*9*), with little or no consensus as to the contribution of punctuational changes to evolutionary divergence. The importance of the theory lies in the challenge it poses for classical accounts of how species diverge.

Punctuational evolution has traditionally been studied in the fossil record. However, phylogenetic trees derived from gene-sequence data contain the signatures of past punctuational and gradual evolution and can be used to study their relative contributions to evolutionary divergence (*10*) (Fig. 1). The nodes of a phylogenetic tree record the number of net-speciation events (speciation-extinction) between the root of the tree and the extant species (Fig. 1, A and B). In phylogenies derived from gene-sequence data, the lengths of the branches of the tree record the expected evolutionary divergence between pairs of speciation events, measured in units of nucleotide substitutions. We denote the sum of the branch lengths between the root of the tree and a species as the path length and write this path length as $x = n\beta + g$, where n is the number of nodes along a path, β is the punctuational contribution of speciation to evolution at each node, and g is the gradual contribution to the path, this being the sum of the individual gradual effects in each branch along the path. Both parameters are measured in units of expected nucleotide substitutions per site in the gene-sequence alignment. Under a gradual model of evolution, there is no punctuational effect, $\beta = 0$, and there should be no relationship between x and n (Fig. 1, B and C). If, however, speciation events are associated with bursts of evolution, then $\beta > 0$, and path lengths from the root to the tips of the tree will be correlated with the number of speciation events that occur along that path (Fig. 1, A and C).

We analyzed 122 gene-sequence alignments selected for including a well-characterized and narrow taxonomic range of species (*11*). This acts to control for background differences among species, such as generation times or adaptive radiation of some lineages, that might affect rates of evolution independently of a punctuational effect. For each data set, we derived a Bayesian sample of the posterior distribution of phylogenetic trees (*11*, *12*). We then estimated β from the relationship between x and n in each tree in the posterior sample to account for phylogenetic uncertainty, using a statistical method (*10*, *13*–*15*) that controls for the shared inheritance of branch lengths implied by the phylogeny (Fig. 1)

Using conservative statistical criteria (*11*), we found a significant relationship between nodes and path lengths (i.e., $\beta > 0$) in 57 [46.7 ± 4.5% (±SE)] of the 122 trees. We removed 22 of these data sets with $\beta > 0$ because they suffered from an artifact of phylogeny reconstruction known as the node-density effect, which can produce an apparent relationship between x and n (*10*, *11*, *16*–*18*). This left 35.0 ± 4.8% of the remaining 100 trees with significant effects of punctuational evolution (Fig. 2), rising to 55.8 ± 7.0% for trees above the median size of $n = 28$ taxa. The overall frequency of 35% is similar to that found in the subset of trees in which 50% of the known taxa have been

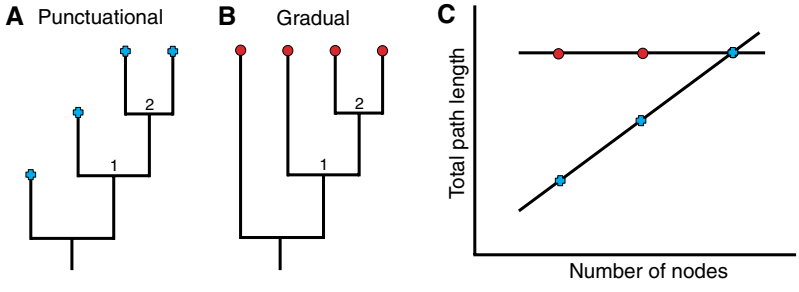


Fig. 1. Signatures of punctuational and gradual evolution on phylogenetic trees. (**A**) Punctuational evolution presumes a burst of evolution associated with each node of the tree. Path lengths, measured as the sum of branches along a path from the root to the tips of the tree, are proportional to the number of nodes along that path (C). Branches are assumed to be in units of nucleotide substitutions. (**B**) Gradual evolution presumes that change is independent of speciation events. Path lengths do not correlate with the number of nodes along a path (C). (**C**) Punctuational evolution predicts a positive relationship between path length and the number of nodes, whereas gradual evolution does not.

School of Biological Sciences, University of Reading, Whiteknights, Reading RG6 6AJ, UK.

*To whom correspondence should be addressed. E-mail: m.pagel@rdg.ac.uk

sampled (28.1 ± 7.95%) and to that in data sets confined to a single genus (32.0 ± 6.60%). However, it is significantly more common in plants (56.7 ± 7.8%) and fungi (71.4 ± 16.0%) than in animals (20.6 ± 4.7%; $\chi^2 = 15.9$, $P < 0.0003$). This difference remains after controlling for the number of taxa in the phylogenetic trees ($P = 0.0063$) and after controlling for the amount of genetic diversity in the phylogeny [plants versus animals, $P = 0.003$; fungi versus animals, $P = 0.008$ (*11*)].

Our principal interest was to estimate the contribution of the punctuational effect at speciation to overall molecular diversity. The mean β is 0.0078 ± 0.0017, ranging from 0.00024 to 0.057, but the absolute values depend on the evolutionary rate of the genes that were used to infer the phylogenetic tree. To control for this, we measured the proportion of the total amount of evolution on the tree attributable to punctuational effects. A bifurcating phylogenetic tree has $2(s - 1)$ branches where s is the number of species. The length of a phylogenetic tree, T, is the sum of the lengths of these branches, here measured in units of nucleotide substitutions. The ratio $2(s - 1)\beta/T$, then, measures the proportion of the tree length attributable to punctuational effects.

If $\beta = 0$, there is no punctuational contribution and the ratio equals zero. If there are no gradual effects, then $2(s - 1)\beta = T$ and the ratio equals 1. We calculated the mean $2(s - 1)\beta/T$ value in the posterior sample of trees derived from each alignment. The mean value of these across the 35 data sets was 0.34 ± 0.08 (median = 0.30); where punctuational effects occurred in our sample, they contributed on average about 30% of the total molecular diversity. This is likely to be an overestimate, because in smaller trees only larger effects will tend to reach statistical significance. Figure 3 plots mean $2(s - 1)\beta/T$ against the number of taxa in the tree for the 35 data sets showing a punctuational effect. The fitted curve (*11*, *19*) has an asymptote as the number of taxa approaches infinity, given by a parameter θ_1, which here takes the value $\hat{\theta}_1 = 0.22 \pm 0.036$. This estimate agrees well with the mean $2(s - 1)\beta/T$ of 0.23 ± 0.12 for trees with greater than the median number of taxa. Even though the frequency of punctuational effects varies among taxonomic groups (Fig. 2), the colored dots of Fig. 3 show that the contribution of punctuational effects to molecular diversity is roughly the same among plants, fungi, and animals.

Punctuational effects cause departures from a molecular clock–like tempo of evolution. By masquerading as gradual changes, punctuational effects will bias estimates of dates derived from molecular clocks that assume gradual evolution, making them occur too far in the past. Departures from the molecular clock are expected to vary with the size of β, as can be seen by studying the expected correlation, ρ, between path lengths and numbers of nodes:

$$\rho = \frac{\beta\sigma_n^2 + \sigma_{g,n}}{\left(\beta^2\sigma_n^2 + \sigma_g^2\right)^{1/2}\left(\sigma_n^2\right)^{1/2}}$$

Here, σ_n^2 is the variance in the number of nodes along the path lengths in the tree, and σ_g^2 is the variance in the gradual effects. The term $\sigma_{g,n}$ denotes the covariance between g, the gradual component of evolution in a path, and n, the number of nodes along the path, and is assumed to be zero. When punctuational effects do not operate, $\beta = 0$, all of the evolutionary change is gradual, and the expected correlation is zero (Fig. 1C). On the other hand, if all of the variation in path lengths arises from the punctuational effect, then $\sigma_g^2 = 0$, and the correlation simplifies to $\rho = \beta\sigma_n^2/\beta\sigma_n^2 = 1$.

Across the 35 data sets showing a punctuational effect, the average correlation be-

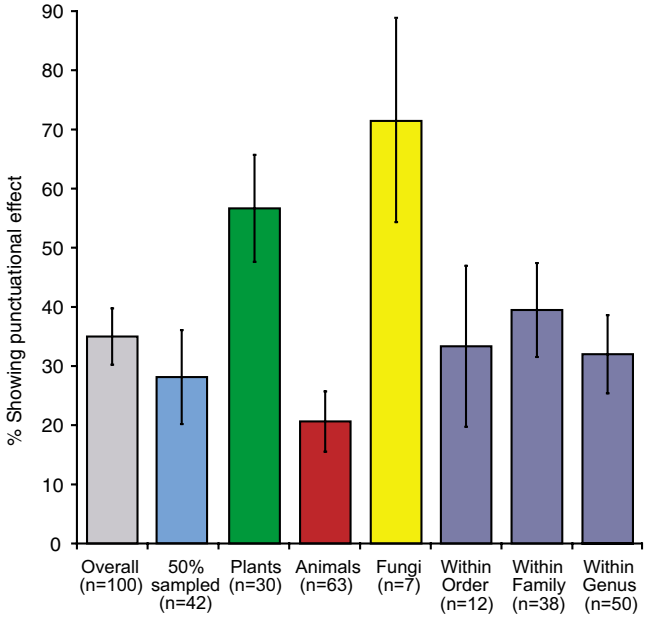


Fig. 2. Percentage of data sets with evidence for punctuational evolution. The Overall data set comprises the 100 trees free of the node-density artifact. 50% sampled refers to the subset of 42 trees in which 50% or more of the known taxa have been sampled. Plants, Animals, and Fungi are the data sets representing these taxonomic groups. Within Order, Within Family, and Within Genus isolate data sets of varying taxonomic range. Apart from differences among taxonomic groups, the rate of punctuational change is relatively stable among the various subsets. Error bars indicate the standard error of the mean.

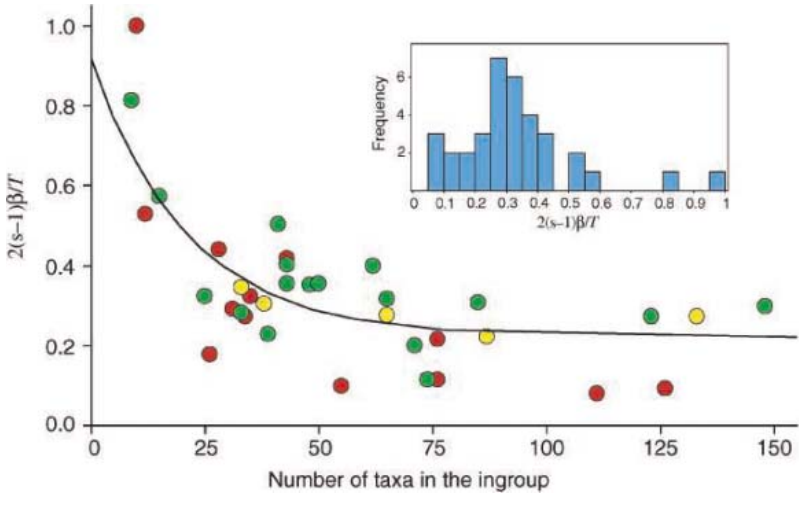


Fig. 3. Relationship between percentage of the tree length attributable to punctuational effects and tree size. Data points plot the mean $2(s - 1)\beta/T$ for each of the 35 phylogenies that show a significant punctuational effect against number of taxa in the tree. One tree, of 10 animal taxa, returned a $2(s - 1)\beta/T > 1.0$. Because this result, apart from sampling error, is impossible, we set this value to 1.0 in the graph and in Fig. 4. As the number of taxa approaches infinity, the curve has an asymptote of 0.22 ± 0.036, indicating that punctuational effects contribute approximately 20% of the total molecular diversity on the tree. Although the rate at which punctuational effects occur is higher in plants and fungi than animals (Fig. 2) the mean contribution to total diversity is similar among groups: plants (green) = 0.31 ± 0.09, animals (red) = 0.36 ± 0.04, fungi (yellow) = 0.28 ± 0.10. The inset displays a frequency histogram of all the $2(s - 1)\beta/T$ values.

tween path lengths and nodes ranges from 0.22 to 0.69 with a mean of $r = 0.42 \pm 0.019$; punctuational effects contributed between 4.8 and 48% (mean $r^2 = 0.42^2 = 0.18$) of the deviation from molecular clock–like behavior in these trees. The remaining variation in path lengths is attributable to variance in gradual effects that is independent of speciation. There is a close correspondence between these r^2 values and the size of the punctuational contribution, as measured by $2(s - 1)\beta/T$. Trees with a larger punctuational component show stronger systematic departures from clock-like evolution (Fig. 4, $r = 0.79$, $P < 0.0001$). When phylogenetic trees derived from gene-sequence data are used to estimate divergence times, punctuational evolution should be tested for and statistically removed before inferring dates.

Punctuational effects appear to be widespread and common. Alternatively, we might be detecting lineages with intrinsically higher rates of evolution that speciate more as a result; this could produce apparent punctuational effects in the form of $\beta > 0$. We think this explanation is unlikely because it has proved difficult to find traits associated with rates of evolution, apart from generation time in a small number of cases (*20*), and these lineage effects would have to be widespread to explain our results. The narrow taxonomic ranges of most of our data sets also mean that the species will have similar morphologies and life histories. A related possibility is that rather than measuring a burst of evolution, we are detecting a generalized increase in the rate of evolution after speciation. This explanation depends on the elevated rate persisting through time and over many branches of the tree. If the rate declined to background between speciation events but was assumed to be "recharged" by successive events of speciation, the phenomenon becomes indistinguishable from punctuational change. Elevated rates associated with lineages or speciation also fail to explain why we found such large differences among taxonomic groups.

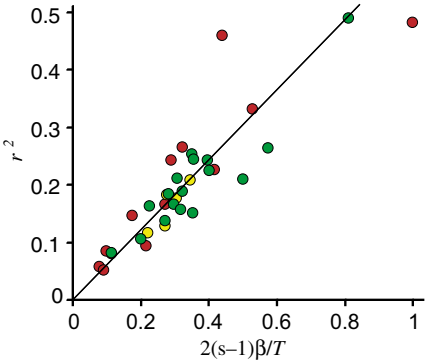


Fig. 4. Relationship of punctuational effects to divergence from the molecular clock. Plot of the correlation between path length and number of nodes (r^2) versus $2(s - 1)\beta/T$ values. The r^2 values record the strength of the relationship between path lengths and numbers of nodes under a punctuational model (Fig. 1); r^2 is expected to be zero when evolution is gradual. The plot shows that when the punctuational contribution is large, as given by $2(s - 1)\beta/T$, there is greater deviation from clock-like behavior.

The punctuational effect we found occurs across a range of genetic loci. If speciation is associated with small founder populations and if genetic isolation is maintained, evolutionary rates can be accelerated at potentially all loci, because the number of loci with alleles governed by drift (neutral plus nearly neutral) is increased (*21*). A second general mechanism for divergence is adaptive evolution as species invade new niches (*22*–*25*). Adaptive divergence is not confined to single loci. In a recent whole-genome comparison of 13,454 human genes with chimpanzee homologs, 71% diverged at coding positions, and 92% diverged at noncoding positions (*26*); this is in two species whose average genetic divergence among homologous genes is about 1%.

Isolating mechanisms that reduce matings between an incipient species population and the ancestral population preserve founder effects and allow adaptive divergence. There is a growing appreciation that sympatric speciation is far more common than previously believed (*27*) and that it arises from specific and often rapid isolating mechanisms operating in small groups. These include mutational changes to signaling molecules or behavioral pathways (*28*–*30*), pollinator switching (*31*), and cytoplasmic incompatibility (*32*). In addition to these, chromosomal rearrangements, changes of ploidy, and hybridization also produce small populations that are frequently unable to mate with the ancestral population. Comparisons of sister species that differ in ploidy suggest that evolution is often accelerated in the polyploids (*33*). These mechanisms could also explain the taxonomic differences we observed in the frequency of punctuational evolution, which is far more common in plants and possibly in fungi than in animals (*34*).

Whatever the mechanisms of the effects we have characterized, relatively rapid and punctuational bursts of evolution driven by speciation appear to make a substantial contribution to molecular divergence. By comparison, we found no molecular counterpart to the periods of stasis noted for morphological traits (*1*, *3*, *4*, *35*, *36*), the other half of the conventional punctuated-equilibrium description of morphological evolution. There need not be any conflict between these two observations as it is well known that molecular change can occur independently of morphology. Punctuational effects are an area of great potential for future research on speciation combining functional-genomic, phylogenetic, physiological, behavioral, and paleontological investigations.

References and Notes

1. N. Eldredge, *Evolution Int. J. Org. Evolution* **25**, 156 (1971).
2. N. Eldredge, S. J. Gould, in *Models in Paleobiology*, T. J. M. Schopf, J. M. Thomas, Eds. (Freeman, Cooper, and Co., San Francisco, CA, 1972), pp. 82–115.
3. N. Eldredge *et al.*, *Paleobiology* **31**, 133 (2005).
4. S. J. Gould, N. Eldredge, *Nature* **366**, 223 (1993).
5. R. E. Lenski, M. Travisano, *Proc. Natl. Acad. Sci. U.S.A.* **91**, 6808 (1994).
6. B. A. Malmgren, W. A. Berggren, G. P. Lohmann, *Science* **225**, 317 (1984).
7. F. H. Rhodes, *Nature* **305**, 269 (1983).
8. R. E. Ricklefs, *Syst. Biol.* **55**, 151 (2006).
9. M. K. Hecht, A. Hoffman, in *Oxford Surveys in Evolutionary Biology*, R. Dawkins, M. Ridley, Eds. (Oxford Univ. Press, Oxford, 1986), pp. 1–48.
10. A. J. Webster, R. J. Payne, M. Pagel, *Science* **301**, 478 (2003).
11. Materials and methods are available as supporting material on *Science* Online.
12. M. Pagel, A. Meade, *Syst. Biol.* **53**, 571 (2004).
13. M. Pagel, *Zool. Scr.* **26**, 331 (1997).
14. M. Pagel, *Nature* **401**, 877 (1999).
15. Software BayesContinuous to calculate punctuational effects is available online (www.evolution.reading.ac.uk), or trees can be submitted to www.evolution.reading.ac.uk/pe to calculate the punctuational effects online.
16. W. M. Fitch, J. J. Beintema, *Mol. Biol. Evol.* **7**, 438 (1990).
17. W. M. Fitch, M. Bruschi, *Mol. Biol. Evol.* **4**, 381 (1987).
18. C. Venditti, A. Meade, M. Pagel, *Syst. Biol.* **55**, 637 (2006).
19. N. R. Draper, H. Smith, *Applied Regression Analysis* (Wiley, New York, ed. 3, 1981).
20. S. Kumar, *Nat. Rev. Genet.* **6**, 654 (2005).
21. T. Ohta, *Annu. Rev. Ecol. Syst.* **23**, 263 (1992).
22. D. N. Reznick, F. H. Shaw, F. H. Rodd, R. G. Shaw, *Science* **275**, 1934 (1997).
23. J. N. Thompson, *Trends Ecol. Evol.* **13**, 329 (1998).
24. R. B. Huey, G. W. Gilchrist, M. L. Carlson, D. Berrigan, L. Serra, *Science* **287**, 308 (2000).
25. C. D. Thomas *et al.*, *Nature* **411**, 577 (2001).
26. The Chimpanzee Sequencing and Analysis Consortium, *Nature* **437**, 69 (2005).
27. E. Culotta, E. Pennisi, *Science* **310**, 1878 (2005).
28. M. Barluenga, K. N. Stolting, W. Salzburger, M. Muschick, A. Meyer, *Nature* **439**, 719 (2006).
29. S. Bearhop *et al.*, *Science* **310**, 502 (2005).
30. G. Gibson, *Science* **307**, 1890 (2005).
31. K. M. Kay, P. A. Reeves, R. G. Olmstead, D. W. Schemske, *Am. J. Bot.* **92**, 1899 (2005).
32. A. Telschow, P. Hammerstein, J. H. Werren, *Evolution Int. J. Org. Evolution* **59**, 1607 (2005).
33. D. E. Soltis, P. S. Soltis, J. A. Tate, *New Phytol.* **161**, 173 (2004).
34. J. A. Coyne, H. A. Orr, *Speciation* (Sinauer Associates, Sunderland, 2004).
35. S. J. Gould, N. Eldredge, *Paleobiology* **3**, 115 (1977).
36. J. B. Jackson, A. H. Cheetham, *Trends Ecol. Evol.* **14**, 72 (1999).

37. This work was supported by the Biotechnology and Biological Sciences Research Council grant G19848 and National Environment Research Council grant H30606 to M.P.

Supporting Online Material

www.sciencemag.org/cgi/content/full/314/5796/119/DC1
Materials and Methods
Table S1
References

8 May 2006; accepted 30 August 2006
10.1126/science.1129647

Action of TFII-I Outside the Nucleus as an Inhibitor of Agonist-Induced Calcium Entry

Gabriela Caraveo,[1] Damian B. van Rossum,[2] Randen L. Patterson,[2*] Solomon H. Snyder,[2,3,4] Stephen Desiderio[1†]

TFII-I is a transcription factor and a target of phosphorylation by Bruton's tyrosine kinase. In humans, deletions spanning the *TFII-I* locus are associated with a cognitive defect, the Williams-Beuren cognitive profile. We report an unanticipated role of TFII-I outside the nucleus as a negative regulator of agonist-induced calcium entry (ACE) that suppresses surface accumulation of TRPC3 (transient receptor potential C3) channels. Inhibition of ACE by TFII-I requires phosphotyrosine residues that engage the SH2 (Src-homology 2) domains of phospholipase C–γ (PLC-γ) and an interrupted, pleckstrin homology (PH)–like domain that binds the split PH domain of PLC-γ. Our observations suggest a model in which TFII-I suppresses ACE by competing with TRPC3 for binding to PLC-γ.

Receptor tyrosine kinases (RTKs) and G protein (heterotrimeric guanine nucleotide–binding protein)–coupled receptors (GPCRs) initiate intracellular calcium signaling by activating γ or β isoforms of PLC (*1*, *2*). The subsequent increase in intracellular calcium involves release of calcium from intracellular stores and calcium entry through transient receptor potential (TRP) channels (*3*, *4*). PLC catalyzes the generation of inositol-1,4,5-trisphosphate (IP3), which triggers calcium release. PLC-γ also promotes ACE by stimulating surface expression of TRPC3 channels; this function requires binding of a partial PH domain in PLC-γ1 to a complementary, PH-like half domain in TRPC3 (*5*, *6*). Because agonist responsiveness can be sensitive to the amplitude and duration of calcium entry (*7*), modulators of ACE may have important physiological roles.

TFII-I is a widely expressed transcription factor originally identified by its interaction with Inr promoter elements (*8*). TFII-I was also identified as a substrate of the Tec-family Bruton's tyrosine kinase, Btk (*9*), which regulates receptor-induced calcium entry in B cells (*10*). Together, TFII-I and Btk activate Inr-dependent transcription, but other kinases, including members of the Src family, also regulate TFII-I (*8*). TFII-I contains six helix-loop-helix (HLH)–like domains, and alternative splicing produces four isoforms (*8*, *11*). In humans, the gene for TFII-I is located on chromosome 7q11.23, in an interval that is invariably deleted in patients with Williams-Beuren syndrome. This multisystemic, contiguous gene disorder includes a severe cognitive defect and a characteristic personality marked by generalized anxiety, empathy, and sociability (*12*, *13*).

Although the transcriptional function of TFII-I implies activity in the nucleus, TFII-I is abundant in the cytosol, as evident from its presence throughout the dendritic tree of cerebellar Purkinje cells (*14*). Indeed, whereas antigenic and growth factor stimulations are

[1]Department of Molecular Biology and Genetics, Institute for Cell Engineering, [2]Department of Neuroscience, [3]Pharmacology and Molecular Science, [4]Psychiatry and Behavioral Sciences, Johns Hopkins University School of Medicine, Baltimore, MD 21205, USA.

*Present address: Department of Biology, Pennsylvania State University, State College, PA 16802, USA.
†To whom correspondence should be addressed. E-mail: sdesider@jhmi.edu

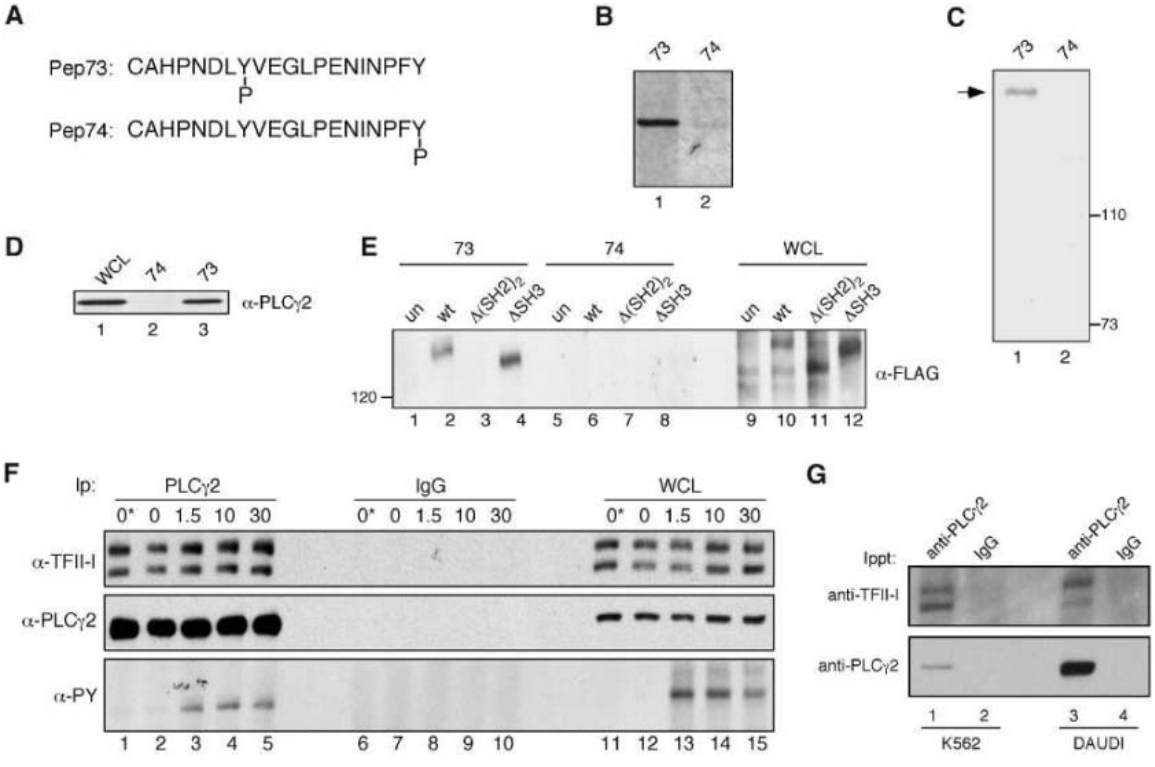


Fig. 1. Specific binding of PLC-γ to a TFII-I tyrosine phosphopeptide. (**A**) Sequences of peptide 73, corresponding to a region spanning the phosphorylation site at Y462, and control peptide 74. (**B**) Peptide-bound proteins from a Ramos B cell lysate were fractionated by SDS–polyacrylamide gel electrophoresis and detected by silver staining. (**C**) Large-scale purification yielded a protein of similar size to that in (B) from beads carrying peptide 73, as detected by Coomasie Blue stain. (**D**) Specific binding of PLC-γ2 to peptide 73 was confirmed by immunoblotting; WCL, whole cell lysate. (**E**) FLAG-tagged wild-type (wt) and mutant PLC-γ1 proteins were expressed in PC12 cells, and lysates were incubated with peptide beads. Proteins bound to peptide 73 or 74 were detected by immunoblotting. Un, untransfected. (**F**) PLC-γ2 was immunoprecipitated (Ip) from the Daudi B cell line before and after B cell receptor cross-linking; precipitates were immunoblotted for TFII-I. Minutes of stimulation are indicated at top; 0* indicates F(ab')2 added after lysis. (**G**) PLC-γ2 was immunoprecipitated (Ippt) from the Daudi or from the K562 hematopoietic cell line, and precipitates were assayed by immunoblotting for TFII-I. IgG, immunoglobulin G.

associated with an increase in the amount of nuclear TFII-I, the cytosolic fraction remains substantial (*15*, *16*). We showed that TFII-I functions in the cytoplasm as a negative regulator of ACE.

TFII-I is phosphorylated in vivo at Tyr^{357} (Y357) and Tyr^{462} (Y462) (*17*), which reside at analogous positions in the loop regions of tandem HLH-like domains (fig. S1A). Because the regions surrounding Y357 and Y462 are nearly identical, we used a phosphopeptide ligand spanning Y462 to identify proteins that interact with one or both sites. The affinity ligand contained an additional, unmodified tyrosine at the C terminus; as a control, we assayed binding to a peptide in which the C-terminal tyrosine residue was phosphorylated instead of the correct internal tyrosine (Fig. 1A).

From a lysate of human B cells, a species of 140 kD bound the correctly phosphorylated peptide but not the control peptide (Fig. 1B). Large-scale purification (Fig. 1C) allowed us to identify the retained protein as PLC-γ2 by mass spectroscopy (Materials and Methods), as confirmed by immunoblotting (Fig. 1D). The broadly expressed isoform PLC-γ1 also showed specific phosphorylation-dependent binding to the Y462 peptide ligand; both PLC-γ isoforms showed phosphorylation-dependent binding to the Y357 peptide ligand (Fig. 1E and fig. S1, B to D). PLC-γ2 and TFII-I associate with each other in vivo, because these proteins were coprecipitated from the B cell line Daudi before and after B cell receptor cross-linking (Fig. 1F); under either condition TFII-I was phosphorylated on tyrosine (fig. S1B). Endogenous PLC-γ2 and TFII-I were also coprecipitated from a lysate of the hematopoietic cell line K562 (Fig. 1G).

We asked whether binding of the TFII-I Y462 phosphopeptide required the Src homology 2 (SH2) domains of PLC-γ. In lysates of cells in which expression of FLAG-tagged PLC-γ1 mutants was induced by removal of doxycycline (*18*), the correctly phosphorylated ligand bound wild-type PLC-γ1 and a PLC-γ1 mutant (ΔSH3) from which the SH3 domain was deleted, but deletion of the SH2 domains [$\Delta(SH2)_2$] abolished specific binding (Fig. 1E).

The association of TFII-I with PLC-γ provoked us to ask whether TFII-I might also modulate intracellular calcium. To test this, we chose the PC12 cell line for its abundant expression of TFII-I and PLC-γ, its ability to mobilize calcium in response to agonists, and its high transfection efficiency. We interrupted TFII-I function with specific small interfering RNA (siRNA) molecules. All isoforms were depleted by 2 days after transfection of siRNA specific for the rat transcript (Fig. 2A). Cells were loaded with the calcium-sensitive dye Fura-2 and stimulated by the GPCR agonists uridine triphosphate (UTP) or bradykinin (*5*, *19*).

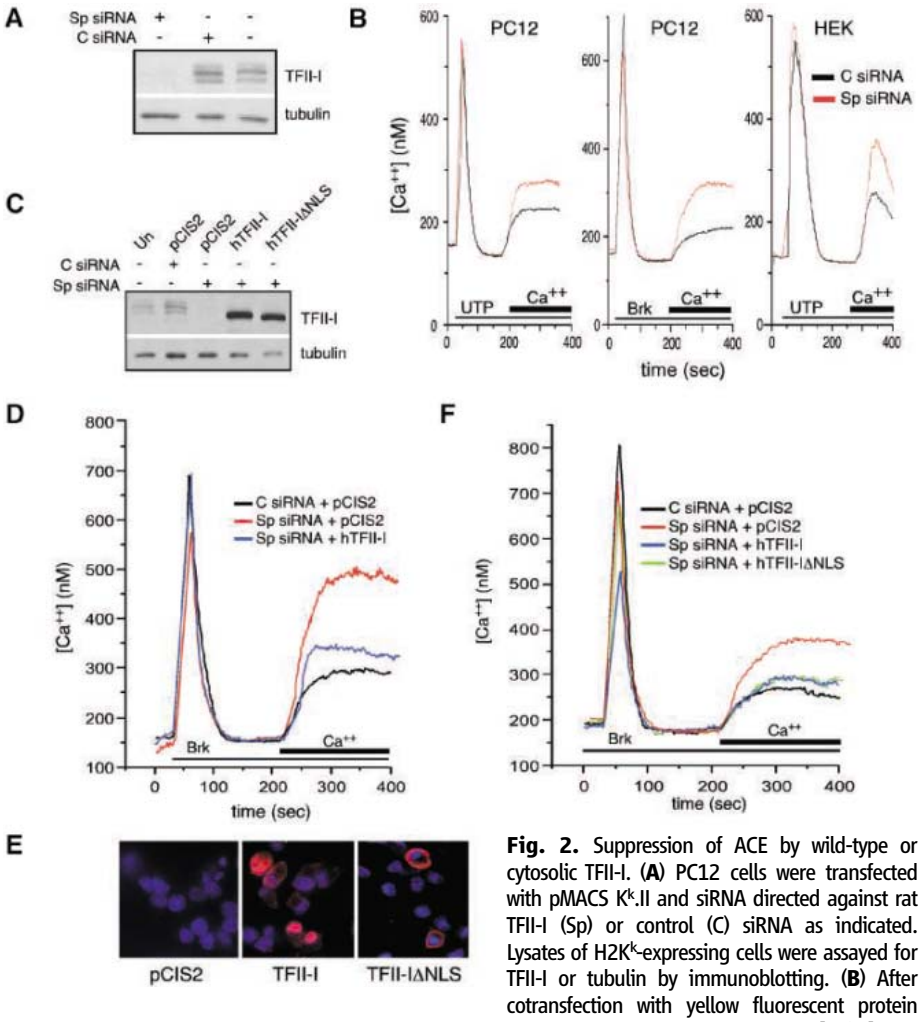


Fig. 2. Suppression of ACE by wild-type or cytosolic TFII-I. (**A**) PC12 cells were transfected with pMACS K^k.II and siRNA directed against rat TFII-I (Sp) or control (C) siRNA as indicated. Lysates of $H2K^k$-expressing cells were assayed for TFII-I or tubulin by immunoblotting. (**B**) After cotransfection with yellow fluorescent protein (YFP) and Sp or C siRNA, PC12 and HEK cells were stimulated with UTP or bradykinin, and $[Ca^{2+}]_i$ was measured in YFP-positive cells. Sp siRNA in HEK cells was directed against human TFII-I. Extracellular calcium was present at times indicated (Ca^{++}). (**C**) PC12 cells were transfected with pMACS K^k.II and Sp siRNA, C siRNA, pCIS2, human TFII-I, or human TFII-IΔNLS, as indicated. $H2K^k$-expressing cells were assayed for TFII-I or tubulin by immunoblotting. (**D**) Representative calcium traces of bradykinin-stimulated PC12 cells cotransfected with YFP, C siRNA, and pCIS2; YFP, Sp siRNA, and pCIS2; or YFP, Sp siRNA, and human TFII-I. (**E**) TFII-I and TFII-IΔNLS were detected in transfected cells by immunofluorescence (rhodamine) and confocal microscopy; nuclei were stained with 4′,6′-diamidino-2-phenylindole. (**F**) Representative calcium traces of bradykinin-stimulated PC12 cells transfected as in (D) or with YFP, Sp siRNA, and human TFII-IΔNLS.

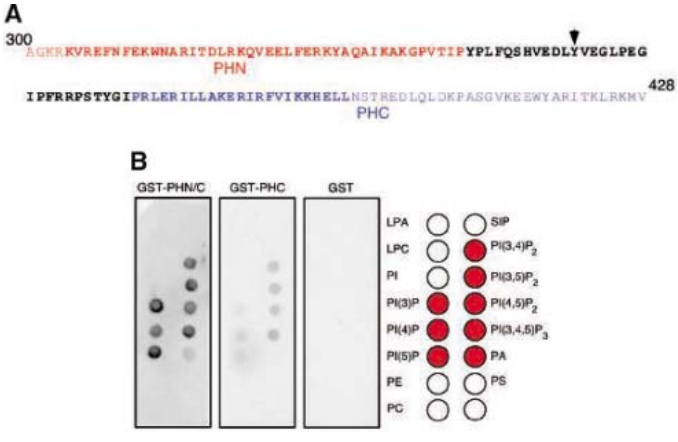


Fig. 3. A split PH-like domain of TFII-I interacts with PLC-γ. (**A**) Sequence of the split PH-like domain identified in figs. S5A and S6. N-terminal (PHN_{like}) portion, red; C-terminal (PHC_{like}) portion, blue. Bold letters, HLH-like repeat sequence. Arrowhead, tyrosine phosphorylation site. (**B**) GST fusions to the PH-like domain or the PHC_{like} portion, and GST alone, were tested for binding to membrane-immobilized phospholipids identified at right and in fig. S3. Bound protein was detected by immunoblotting for GST.

We stimulated cells in the absence of extracellular calcium and recorded release of intracellular calcium. After free intracellular calcium ($[Ca^{2+}]_i$) returned to basal concentrations, we introduced extracellular calcium in the presence of agonist and recorded calcium entry. Depletion of TFII-I was associated with increased calcium entry in cells stimulated with UTP or bradykinin (Fig. 2B). This effect was also observed in human embryonic kidney (HEK) cells with siRNA against human TFII-I (Fig. 2B). The human TFII-I transcript diverges from the rat transcript in the region targeted by the siRNA used to deplete TFII-I from PC12 cells. Exploiting this divergence, we observed that human TFII-I isoform 4, which was expressed in the presence of rat-specific siRNA (Fig. 2C), rescued the calcium phenotype (Fig. 2D). These results suggested that TFII-I is a negative regulator of ACE.

The effect of TFII-I on calcium entry could have resulted from altered expression of TFII-I–dependent genes or from a nontranscriptional function. To eliminate the transcriptional function of TFII-I, we deleted the nuclear localization signal (TFII-IΔNLS) (*11*). TFII-IΔNLS was absent from the nucleus (Fig. 2E) and lacked transcriptional activity, as assessed by using the *c-fos* promoter (*20*) (fig. S2). Wild-type human TFII-I and TFII-IΔNLS were expressed at similar amounts in siRNA-treated cells (Fig. 2C) and were similarly efficient in rescuing the calcium entry phenotype (Fig. 2, F and G, and fig. S3).

In some experiments, TFII-I depletion suppressed release of intracellular calcium. For several reasons, it seems unlikely that the effect of TFII-I depletion on calcium entry resulted from an effect on calcium release. First, TFII-I depletion consistently stimulated ACE, regardless of the effect on calcium release (fig. S4A). Second, in line with previous studies (*21*–*23*), there was no correlation between agonist-induced calcium release and calcium entry (fig. S4B). Third, depletion of intracellular calcium stores by thapsigargin or ionomycin did not stimulate calcium entry (fig. S4C).

Stimulation of ACE by PLC-γ requires an interaction between a split PH domain in PLC-γ

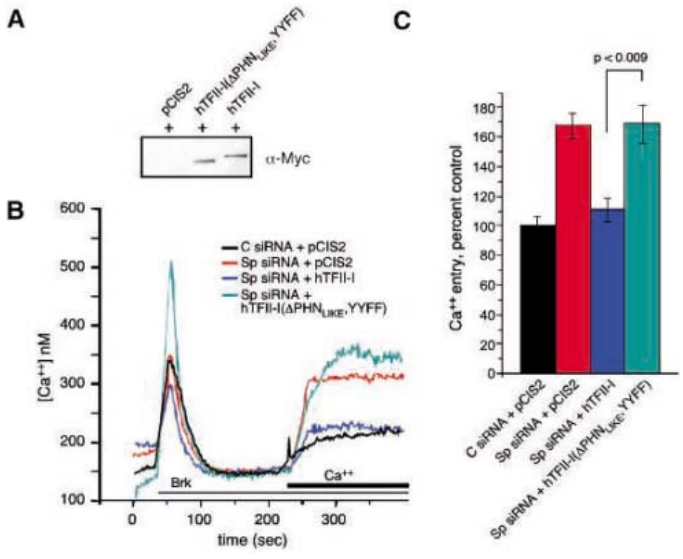


Fig. 4. Failure of TFII-I(ΔPHN_{like},YY/FF) to suppress ACE. (**A**) PC12 cells were transfected with a plasmid encoding myc-tagged human (h) TFII-I or myc-tagged TFII-I(ΔPHN_{like},YY/FF) or with pCIS2 and assayed by immunoblotting for Myc. (**B**) Representative calcium traces of bradykinin-stimulated PC12 cells cotransfected with YFP, control (C) siRNA, and pCIS2; YFP, specific (Sp) siRNA, and pCIS2; YFP, Sp siRNA, and human TFII-I; or YFP, Sp siRNA, and human TFII-I(ΔPHN_{like},YY/FF). (**C**) Calcium entry, defined as the integral of $[Ca^{++}]_i$ over the time interval of 200 to 400 s, was normalized to control. Mean and SEM are plotted for 350 cells from each transfected cell population in (B). Difference between wild type and TFII-I(ΔPHN_{like},YY/FF) estimated as pairwise comparison by using a two-tailed *t* test; one way analysis of variance (ANOVA) yielded similar results.

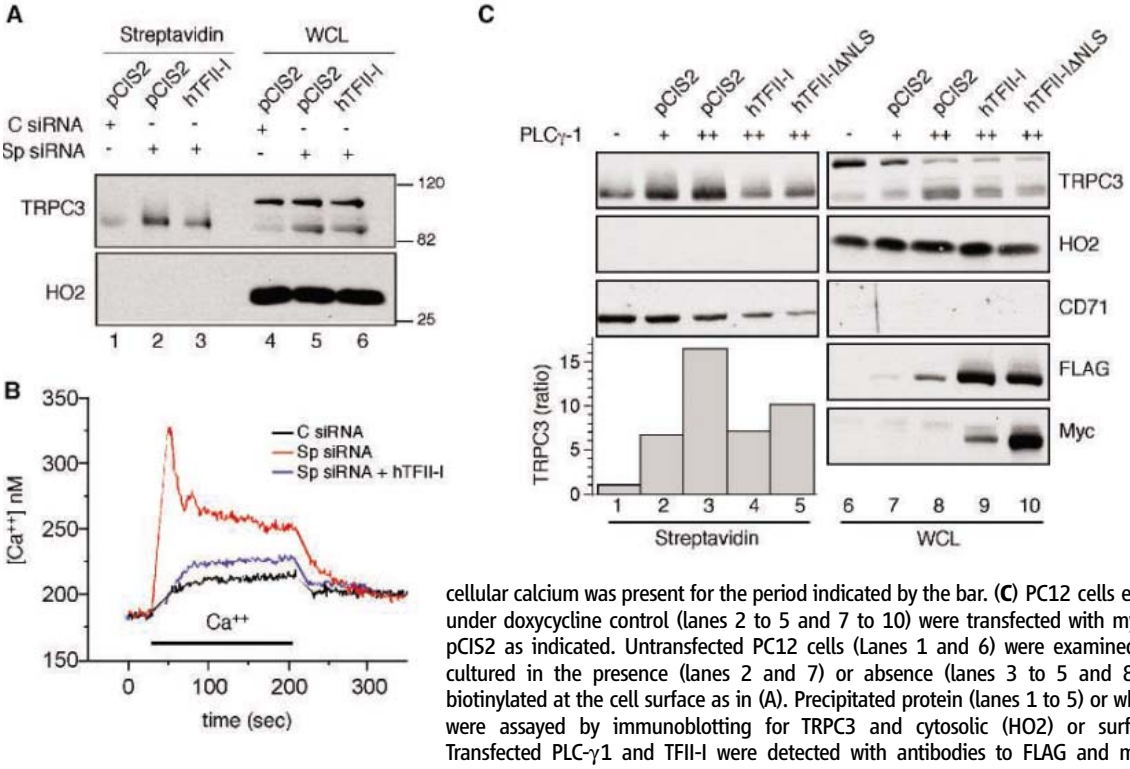


Fig. 5. TRPC3 accumulation after depletion of TFII-I. (**A**) PC12 cells were transfected with pMACS K^k.II and specific (Sp) siRNA, control (C) siRNA, pCIS2, and human TFII-I as indicated. $H2K^k$-expressing cells were isolated and subjected to cell surface biotinylation. Biotinylated protein was immunoprecipitated with streptavidin agarose. Precipitated protein (streptavidin IP) or whole cell lysates (WCL) were assayed by immunoblotting for TRPC3 or the cytosolic control protein heme oxygenase 2 (HO2). (**B**) Entry of extracellular calcium was measured without agonist treatment in PC12 cells transfected as in Fig. 2C. Extracellular calcium was present for the period indicated by the bar. (**C**) PC12 cells expressing FLAG-tagged PLC-γ1 under doxycycline control (lanes 2 to 5 and 7 to 10) were transfected with myc-tagged TFII-I, TFII-IΔNLS, or pCIS2 as indicated. Untransfected PC12 cells (Lanes 1 and 6) were examined in parallel. Transfected cells, cultured in the presence (lanes 2 and 7) or absence (lanes 3 to 5 and 8 to 10) of doxycycline, were biotinylated at the cell surface as in (A). Precipitated protein (lanes 1 to 5) or whole cell lysates (lanes 6 to 10) were assayed by immunoblotting for TRPC3 and cytosolic (HO2) or surface (CD71) control proteins. Transfected PLC-γ1 and TFII-I were detected with antibodies to FLAG and myc epitopes, respectively. The histogram indicates the relative ratio of TRPC3 to CD71 in streptavidin precipitates, normalized to the ratio obtained for untransfected PC12 cells.

and a PH-like region of TRPC3 (*6*). The ability of cytosolic TFII-I to modulate calcium entry prompted us to ask whether TFII-I might also contain a PH-like domain that could compete with TRPC3 for binding to PLC-γ. We used a Gestalt domain-detection algorithm (GDDA) that was designed to increase the sensitivity of reversed position-specific BLAST (rps-BLAST) searches (*24*, *25*). Applying the GDDA for PH-like domains in TFII-I, we found a region that overlapped the second HLH-like repeat (fig. S5A). This domain was split by an interval spanning the tyrosine phosphorylation site at Y357 that supports binding to the PLC-γ SH2 region (Fig. 3A and fig. S6).

PH domains often exhibit selective lipid binding (*26*). To extend the computational identification of a PH-like domain in TFII-I, we tagged this region with glutathione S-transferase (GST) and tested for binding to immobilized lipids. The complete PH-like domain from TFII-I showed preferential binding to phosphatidylinositols (Fig. 3B), whereas other lipids that are negatively charged at physiological pH, such as phosphatidyl ethanolamine, phosphatidyl serine, and sphingosine 1-phosphate, showed no detectable binding. Binding was reduced by removal of the N-terminal half of the PH-like domain and was undetectable when GST alone was used as a probe (Fig. 3B). Moreover, the PH-like domain of TFII-I preferred binding di- and triphosphorylated phosphatidylinositols over monophosphorylated species (fig. S5B).

We used a yeast two-hybrid assay to test binding of the TFII-I PH-like domain to the N-terminal (PHN) and C-terminal (PHC) PH half domains of PLC-γ. A fragment spanning the N- and C-terminal halves of the TFII-I PH-like domain (PHN_{like}-L-PHC_{like}, where L denotes the linker joining the N- and C-terminal halves) failed to bind the PLC-γ1 PHC domain, whereas a fragment spanning the N-terminal half domain alone (PHN_{like}) did bind, as assessed by β-galactosidase expression (fig. S5, C and D). In contrast, the TFII-I C-terminal half domain (PHC_{like}) did not bind (fig. S5, C and D). Binding of the PLC-γ1 PHC domain to the TFII-I PHN-like domain could only be detected after deletion of the TFII-I PHC-like domain. If the TFII-I PHN-like and PHC-like domains were removed, an interaction could still be detected between the TFII-I linker region and the PLC-γ1 PHC domain (fig. S5, C and D).

We thus identified two modes of interaction between TFII-I and PLC-γ. To test the contributions of these binding modalities to the modulation of ACE, we tested rescue of the calcium mobilization phenotype in TFII-I–depleted PC12 cells. TFII-I(YY357, 462FF), which lacks PLC-γ SH2 binding sites, exhibited partial rescue of the calcium entry phenotype, as did a mutant that lacks the PHN-like region (fig. S7, A and C). In contrast, the double mutant, TFII-I(ΔPHN_{like},YY/FF), did not restore calcium entry (Fig. 4, B and C), despite the fact that its abundance was similar to that of wild type (Fig. 4A). It is not likely that the debilitating effect of the PHN_{like} deletion was caused by structural disruption of the PH-like domain, because deletion of the PHC_{like} half domain did not impair TFII-I function in the calcium assay (fig. S7, B and C). These results suggest a model in which TFII-I inhibits calcium entry by antagonizing PLC-γ through multiple interactions that are mediated by phosphotyrosine and the PH-like domain.

Because PLC-γ stimulates calcium influx by inducing surface accumulation of TRPC3 calcium channels (*6*), we examined the effect of TFII-I depletion on TRPC3 surface accumulation in PC12 cells. Depletion of TFII-I was associated with increased surface accumulation of TRPC3; this increase could be partially reversed by enforced expression of human TFII-I isoform 4 (Fig. 5A). Although TFII-I depletion was also associated with an increase in total TRPC3 (Fig. 5A), this increase was not accompanied by an increase in the steady-state accumulation of TRPC3 mRNA (fig. S8), suggesting a posttranscriptional effect and in agreement with the ability of cytosolic TFII-I to rescue the calcium entry phenotype. Increased surface expression of TRPC3 could explain the stimulatory effect of TFII-I depletion on calcium entry. Indeed, depletion of TFII-I was associated with constitutive calcium entry, which was reversed by human TFII-I (Fig. 5B). These observations support a mechanism in which TFII-I modulates calcium entry by regulating the density of TRPC3 channels at the cell surface.

We asked whether TFII-I could counteract the ability of PLC-γ to stimulate surface expression of TRPC3 by using a PC12 derivative in which FLAG-tagged PLC-γ is induced by doxycycline removal (*18*). Cells expressing FLAG-PLC-γ exhibited increased surface expression of TRPC3, and this surface expression increased further upon doxycycline withdrawal (Fig. 5C). Thus, the effect of exogenous PLC-γ on TRPC3 expression was similar to that of TFII-I depletion by siRNA treatment. The stimulatory effect of PLC-γ on TRPC3 accumulation could be reversed by overexpression of Myc-tagged TFII-I or TFII-IΔNLS (Fig. 5C).

We propose a model in which TFII-I fluctuates between "open" and "closed" states (fig. S9). In the open state, which may be stabilized by phosphorylation of TFII-I at Y357 by Btk or other kinases, the TFII-I PHN-like domain and linker region may interact with the split PH domain of PLC-γ, while phospho-Y357 engages PLC-γ through an SH2 domain. This interaction may inhibit calcium flux because TFII-I competes with TRPC3 for binding to PLC-γ. Dephosphorylation of TFII-I would favor the closed state, freeing PLC-γ to stimulate surface accumulation of TRPC3. We considered the possibility that the effect of TFII-I depletion on ACE might reflect altered expression of genes involved in calcium entry. Two lines of evidence argue against this. First, the TFII-IΔNLS mutant is excluded from the nucleus in unstimulated cells, and ACE is measured within 4 min after agonist treatment, faster than expected for a transcriptional response. Second, TFII-IΔNLS failed to stimulate transcription from a TFII-I–responsive reporter. Thus, TFII-I joins c-fos as a transcription factor with a distinct cytosolic function (*27*, *28*). The function of TFII-I as a negative regulator of ACE is interesting given the association of *TFII-I* deletion with the cognitive defects of Williams-Beuren syndrome (*29*). Our results may help define a genetic etiology for this disorder, because defects in calcium transport have been associated with other neurobehavioral syndromes (*30*).

References and Notes

1. M. J. Berridge, *Nature* **361**, 315 (1993).
2. D. E. Clapham, *Cell* **80**, 259 (1995).
3. D. E. Clapham, *Nature* **426**, 517 (2003).
4. C. Montell, K. Jones, E. Hafen, G. Rubin, *Science* **230**, 1040 (1985).
5. R. L. Patterson *et al.*, *Cell* **111**, 529 (2002).
6. D. B. van Rossum *et al.*, *Nature* **434**, 99 (2005).
7. C. Utzny, M. Faroudi, S. Valitutti, *Biophys. J.* **88**, 1 (2005).
8. A. L. Roy, *Gene* **274**, 1 (2001).
9. W. Yang, S. Desiderio, *Proc. Natl. Acad. Sci. U.S.A.* **94**, 604 (1997).
10. A. C. Fluckiger *et al.*, *EMBO J.* **17**, 1973 (1998).
11. V. Cheriyath, A. L. Roy, *J. Biol. Chem.* **275**, 26300 (2000).
12. L. R. Osborne, T. Campbell, A. Daradich, S. W. Scherer, L. C. Tsui, *Genomics* **57**, 279 (1999).
13. L. A. Perez Jurado *et al.*, *Hum. Mol. Genet.* **7**, 325 (1998).
14. S. K. Danoff, H. E. Taylor, S. Blackshaw, S. Desiderio, *Neuroscience* **123**, 931 (2004).
15. V. Cheriyath, Z. P. Desgranges, A. L. Roy, *J. Biol. Chem.* **277**, 22798 (2002).
16. C. D. Novina *et al.*, *Mol. Cell. Biol.* **19**, 5014 (1999).
17. A. M. Egloff, S. Desiderio, *J. Biol. Chem.* **276**, 27806 (2001).
18. K. Ye *et al.*, *Nature* **415**, 541 (2002).
19. K. Venkatachalam, H. T. Ma, D. L. Ford, D. L. Gill, *J. Biol. Chem.* **276**, 33980 (2001).
20. D. W. Kim, V. Cheriyath, A. L. Roy, B. H. Cochran, *Mol. Cell. Biol.* **18**, 3310 (1998).
21. M. C. Hermosura *et al.*, *Nature* **408**, 735 (2000).
22. C. M. Peppiatt *et al.*, *Biochem. J.* **381**, 929 (2004).
23. D. B. van Rossum *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **101**, 2323 (2004).
24. A. Marchler-Bauer, S. H. Bryant, *Nucleic Acids Res.* **32**, W327 (2004).
25. R. L. Patterson, D. B. van Rossum, N. Nikolaidis, D. L. Gill, S. H. Snyder, *Trends Biochem. Sci.* **30**, 688 (2005).
26. M. A. Lemmon, K. M. Ferguson, *Biochem. Soc. Trans.* **29**, 377 (2001).
27. D. F. Bussolino *et al.*, *FASEB J.* **15**, 556 (2001).
28. G. A. Gil *et al.*, *Mol. Biol. Cell* **15**, 1881 (2004).
29. H. Hirota *et al.*, *Genet. Med.* **5**, 311 (2003).
30. I. Splawski *et al.*, *Cell* **119**, 19 (2004).

31. We thank R. Barrow, M. Mauer, and A. M. Egloff for assistance and our colleagues in the Department of Molecular Biology and Genetics for stimulating discussions. Supported by the Howard Hughes Medical Institute, a grant from the National Cancer Institute, and an anonymous gift in support of the Institute for Cell Engineering.

Supporting Online Material

www.sciencemag.org/cgi/content/full/314/5796/122/DC1
Materials and Methods
Figs. S1 to S8

23 March 2006; accepted 3 August 2006
10.1126/science.1127815

Cancer Regression in Patients After Transfer of Genetically Engineered Lymphocytes

Richard A. Morgan, Mark E. Dudley, John R. Wunderlich, Marybeth S. Hughes, James C. Yang, Richard M. Sherry, Richard E. Royal, Suzanne L. Topalian, Udai S. Kammula, Nicholas P. Restifo, Zhili Zheng, Azam Nahvi, Christiaan R. de Vries, Linda J. Rogers-Freezer, Sharon A. Mavroukakis, Steven A. Rosenberg*

Through the adoptive transfer of lymphocytes after host immunodepletion, it is possible to mediate objective cancer regression in human patients with metastatic melanoma. However, the generation of tumor-specific T cells in this mode of immunotherapy is often limiting. Here we report the ability to specifically confer tumor recognition by autologous lymphocytes from peripheral blood by using a retrovirus that encodes a T cell receptor. Adoptive transfer of these transduced cells in 15 patients resulted in durable engraftment at levels exceeding 10% of peripheral blood lymphocytes for at least 2 months after the infusion. We observed high sustained levels of circulating, engineered cells at 1 year after infusion in two patients who both demonstrated objective regression of metastatic melanoma lesions. This study suggests the therapeutic potential of genetically engineered cells for the biologic therapy of cancer.

In the past two decades, fundamental advances in immunology have introduced opportunities for the development of cellular-based therapies for the treatment of cancer (*1*, *2*). After ex vivo expansion, transfer, and clonal repopulation in patients who have received lymphodepleting conditioning, autologous tumor-infiltrating lymphocytes (TILs) have been found to mediate objective cancer regression in a measurable proportion of patients with metastatic melanoma (*3*–*5*). A limitation of this approach is the requirement that patients have preexisting tumor-reactive cells that can be expanded ex vivo. In addition, in many cancer patients, especially those with cancers other than melanoma, it is difficult to identify these tumor-reactive lymphocytes. To overcome this limitation, we set out to develop an approach to cancer immunotherapy based on the genetic modification of normal peripheral blood lymphocytes (PBLs).

Tumor-associated antigens (TAAs) are recognized by the T cell receptor (TCR) on the T lymphocyte surface, which is composed of the TCR alpha and beta chains (*6*). The genes encoding the TCR that are specific for a variety of TAA have now been cloned, including the TCR-recognizing MART-1 and gp100 melanoma/melanocyte differentiation antigens, the NY-ESO-1 cancer-testis antigen that is present on many common epithelial cancers, and an epitope from the p53 molecule, which is expressed on the surface of approximately 50% of cancers of common epithelial origin (*7*–*12*). In each case, these antigens were detected by the TCR when they were presented as peptides by molecules encoded by the major histocompatibility complex protein human lymphocyte antigen (HLA)–A2. In vitro transcribed RNA from four TAA-reactive TCRs (recognizing MART-1:27–35, gp100:209–217, NY-ESO-1:157–165, and p53:264–272) were electroporated into $CD8^+$ PBLs, which were then cocultured with peptide-pulsed T2 cells. These transfected cells produced large amounts of interferon-γ (IFN-γ) upon stimulation with their respective peptides (Fig. 1A) and were able to recognize HLA-A2–matched tumors, including melanoma, lung cancer, and breast cancer (table S1). Furthermore, transduction with these TCR-encoding retroviral vectors converted normal PBLs into cells capable of specifically recognizing and destroying both fresh and cultured cells from multiple common cancers (such as sarcoma and breast, lung, esophagus, and liver cancers) in vitro (*9*–*12*).

Surgery Branch, Center for Cancer Research, National Cancer Institute, National Institutes of Health, 10 Center Drive, Bethesda, MD 20892, USA.

*To whom correspondence should be addressed. E-mail: SAR@mail.nih.gov

Table 1. Patient demographics, treatments received, and clinical outcome. Ln, lymph node; Cu, cutaneous; Sub, subcutaneous; Li, liver; Lu, lung; Ad, adrenal; Pa, pancreas; Br, brain; Hi, hilum. NR, no response; PR, partial response; MR, minor or mixed response.

Cohort	Patient	Age/sex	Total cells infused ($\times 10^{-9}$)	CD4/CD8 (%)	VB12 (%)	MART-1 cells infused ($\times 10^{-9}$)‡	Days in culture	Doubling time (days)†	IL-2 doses§	Sites of evaluable disease	Response (duration in months)‖
1	1	28/M	11.0	27/73	67	7.4	19	8.7	7	Ln, Cu	NR
	2a*	44/F	13.0	3/95	64	8.3	19	11.9	10	Ln, Cu	NR
	3	58/M	14.0	17/82	35	4.9	19	10.0	11	Cu, Sub	NR
2	4	52/M	1.0	50/50	42	0.5	6	1.4	9	Li, Sub	PR(21)
	5	50/M	12.0	18/82	17	2.2	8	1.0	7	Lu, Ln, Sub	NR
	6	55/F	7.0	37/72	51	3.6	7	1.3	8	Lu, Ln	NR
	7	56/M	9.0	75/21	40	3.6	7	1.0	5	Lu, Ln	NR
	8	37/M	6.1	68/40	32	1.9	7	1.3	12	Lu, Ln	NR
	9	53/M	4.2	72/24	41	1.7	7	2.0	9	Ln, Ad, Sub	MR
	10	45/M	8.6	53/30	34	2.9	6	0.6	5	Ln, Sub	
	11	45/M	6.3	7/92	45	2.8	6	0.8	5	Lu, Pa, Ln	NR
	12	32/F	4.7	30/60	61	2.9	6	0.7	5	Br, Sub	NR
	13	41/M	7.7	40/67	42	3.2	6	0.9	7	Lu, Sub	NR
	2b*	44/F	2.1	30/59	53	1.1	6	1.9	14	Ln, Cu	NR
3	14	30/M	86	11/60	40	34.4	18+9	0.9	5	Hi	PR(20)
	15	51/M	38	16/82	45	17.1	18+9	3.3	8	Lu	NR
	16	25/F	33	13/76	21	6.9	18+9	1.2	2	Lu, Li, Sub	NR
	17	20/F	23	17/78	30	6.9	17+8	1.1	3	Lu, Ln, Sub	NR

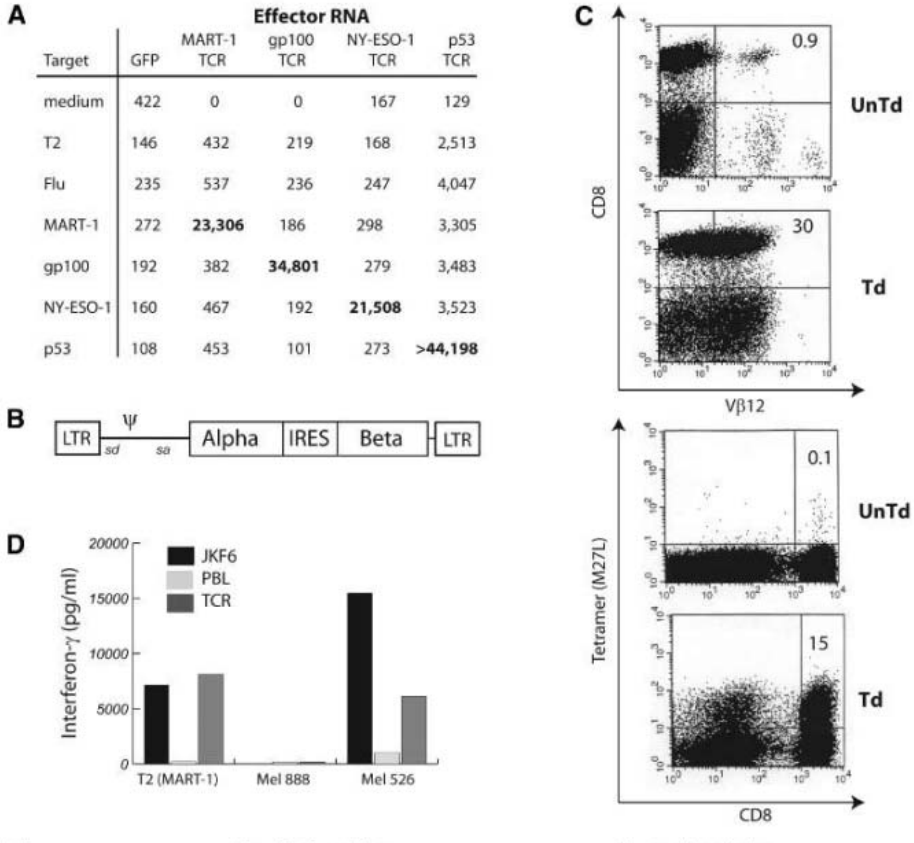
*This patient was treated twice; treatments were separated by 7 months. †Determined based on cell counts in the 2 days before infusion. ‡Total cells infused multiplied by %Vβ12. §720,000 international units/kg every 8 hours. All patients were previously refractory to treatment with IL-2 alone. ‖Based on RECIST criteria.

To investigate the ability of genetically engineered PBLs to recognize and destroy tumor cells in vivo, we transduced PBLs derived from patients with melanoma with the genes encoding the alpha and beta chains of the anti–MART-1 TCR. These genes were cloned from a TIL clone obtained from a cancer patient who demonstrated a near complete regression of metastatic melanoma after adoptive cell transfer (ACT) of TILs (*5*). A retroviral vector was constructed and optimized to express the MART-1 TCR alpha and beta chains (Fig. 1B) (*13*). Gene transfer efficiency, assessed by staining for the specific Vβ12 protein in this TCR, resulted in expression in 30% of the transduced $CD8^+$ cells (Fig. 1C), as compared with ~1% of untransduced control cell cultures (gene transfer was about equally divided between CD4 and CD8 cells). Fifteen percent of the transduced $CD8^+$ cells bound the MART-1 peptide-specific HLA-A*0201 tetramer (Fig. 1C and table S2). The TCR-transduced cells were biologically active, as demonstrated by the specific secretion of IFN-γ after coculture with both MART-1 peptide-pulsed cells and HLA-A2 positive melanoma cell lines (Fig. 1D).

To investigate the in vivo efficacy of these MART-1 TCR-engineered T cells, we selected 17 HLA-A*0201 patients with progressive metastatic melanoma (Table 1) for treatment. Cancers in all patients were refractory to previous therapy with interleukin-2 (IL-2). T cell cultures from all 17 patients were biologically reactive, with specific secretion of IFN-γ after coculture with either MART-1 peptide-pulsed T2 cells and/or melanoma cell lines expressing the MART-1 antigen (Fig. 1E). Gene transfer efficiencies measured by staining for Vβ12 expression in these lymphocytes ranged from 17 to 67% (42%, mean value) (Table 1 and table S2).

Patients received ACT treatments with MART-1 TCR-transduced autologous PBLs at a time of maximum lymphodepletion (*13*). Three patients in an initial cohort were treated with cells after an extended culture period of 19 days and had cell doubling times ranging from 8.7 to 11.9 days (Table 1, cohort 1, patients 1, 2a, and 3). In these patients, <10% of the transduced cells persisted across the time points tested during the first 30 days after infusion, and ≤2% of the cells persisted beyond 50 days (Fig. 2A). These first three patients showed no delay in the progression of disease.

In an effort to administer gene-modified lymphocytes that were in their active growth phase, the culture conditions were modified (*13*) to limit the ex vivo culture period to between 6 and 9 days after stimulation of cells with antibody to CD3 (Table 1, cohort 2, cell doubling times of ≤2 days). In another cohort, larger numbers of actively dividing cells for ACT were generated by performing a second rapid expansion protocol (*14*) after 8 to 9 days (Table 1, cohort 3, cell doubling times from 0.9 to 3.3 days). In contrast



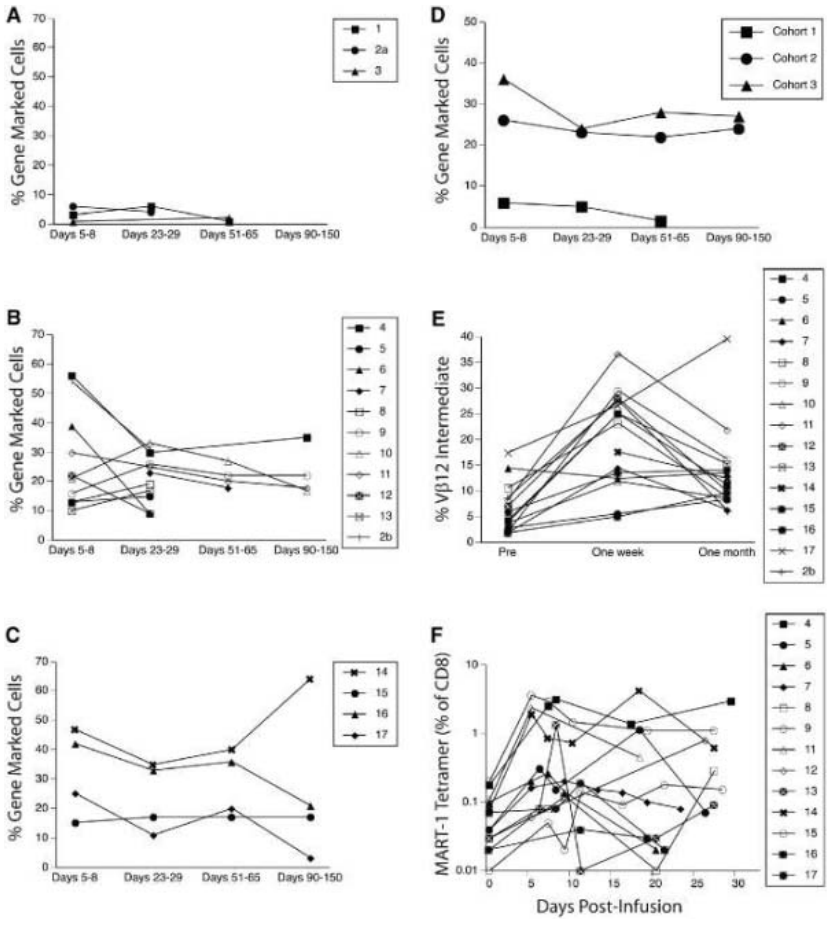
Target	GFP	MART-1 TCR	gp100 TCR	NY-ESO-1 TCR	p53 TCR
medium	422	0	0	167	129
T2	146	432	219	168	2,513
Flu	235	537	236	247	4,047
MART-1	272	**23,306**	186	298	3,305
gp100	192	382	**34,801**	279	3,483
NY-ESO-1	160	467	192	**21,508**	3,523
p53	108	453	101	273	**>44,198**

E

Cohort	Patient	Peptide Reactivity gp100	Peptide Reactivity MART-1	Tumor Reactivity 526	Tumor Reactivity 624	Tumor Reactivity 888	Tumor Reactivity 938
1	1	48	3,095	2,285	862	81	44
	2a	76	691	998	1,870	76	8
	3	66	1,238	345	474	121	50
2	4	588	6,315	3,072	2,200	183	135
	5	406	5,990	2,865	2,595	>2,882	274
	6	1,801	8,135	6,120	1,140	183	108
	7	126	1,698	610	199	142	185
	8	187	2,840	885	687	112	67
	9	113	5,680	751	433	100	91
	10	1,462	>22,045	9,150	7,495	724	656
	11	1,494	>23,975	7,745	4,805	327	275
	12	1,106	>23,450	8,190	4,315	168	131
	13	298	10,285	6,435	6,945	103	80
	2b	118	5,130	4,545	3,155	82	35
3	14	33	2,785	426	263	42	52
	15	213	3,115	964	453	122	115
	16	72	4,210	1,107	683	31	99
	17	148	4,600	1,035	848	50	57

Fig. 1. Transduction and analysis of TCR-engineered cells. (**A**) $CD8^+$ human lymphocytes were electroporated with RNA encoding control [green fluorescent protein (GFP)] or cloned TCRs reactive with HLA-A2 restricted epitopes from the human TAAs MART-1, gp100, NY-ESO-1, and p53. Effector T cells were cocultured with T2 cells pulsed with 1 μM of the indicated peptide (values are expressed as IFN-γ in pg/ml). Values demonstrating the specific release of cytokine are in bold. (**B**) Diagram of the recombinant retroviral vector MSGV1AIB used to engineer human lymphocytes. LTR, long terminal repeat; Ψ, extended packaging signal; sd, splice donor; sa, splice acceptor; Alpha, alpha chain; IRES, internal ribosomal entry site; Beta, beta chain. (**C**) Transduced (Td) lymphocytes were analyzed 5 days after transduction for the expression of Vβ12 and MART-1 tetramer [Ala^{27}→Leu^{27} (A27L)] in $CD8^+$ cells in comparison with untransduced (UnTd) cells. Numbers in the upper-right corners indicate the percentage of positive cells in that quadrant. (**D**) TCR vector-engineered cells from patient 6 (TCR) were cocultured with MART-1 peptide-pulsed T2 cells, HLA-$A2^-$ melanoma line (Mel 888), or HLA-$A2^+$ melanoma line (Mel 526), and the amount of IFN-γ produced was determined. Control effectors were untransduced cells (PBL) and the MART-1–reactive TIL JKF6 (JKF6). (**E**) Anti-melanoma properties of genetically engineered lymphocytes were determined for all patients before infusion. The production of IFN-γ (pg/ml) after coculture with peptide-pulsed T2 cells (Peptide Reactivity) and anti-melanoma activity (Tumor Reactivity) for HLA-$A2^+$ lines (526 and 624) and HLA-$A2^-$ lines (888 and 938).

Fig. 2. Persistence of gene-marked cells. DNA extracted from peripheral blood mononuclear cells (PBMCs) was subjected to real-time quantitative PCR to determine the percentage of vector-transduced cells in patient circulation at various times after infusion. Each line represents data from a separate patient. (**A**) Cohort 1; (**B**) Cohort 2; (**C**) Cohort 3. (**D**) Mean value of the percentage of gene-marked cells for all patients in each cohort at the given time interval after treatment. (**E**) The percentage of $CD8^+$/ $V\beta12^+$ cells in the intermediate gate (*13*) for patients in cohorts 2 and 3 is shown. (**F**) The percentage of $CD8^+$/MART-1^+ tetramer cells was determined for patients in cohorts 2 and 3 at the times shown. Pretreatment values for each patient are plotted as day 0 after infusion.



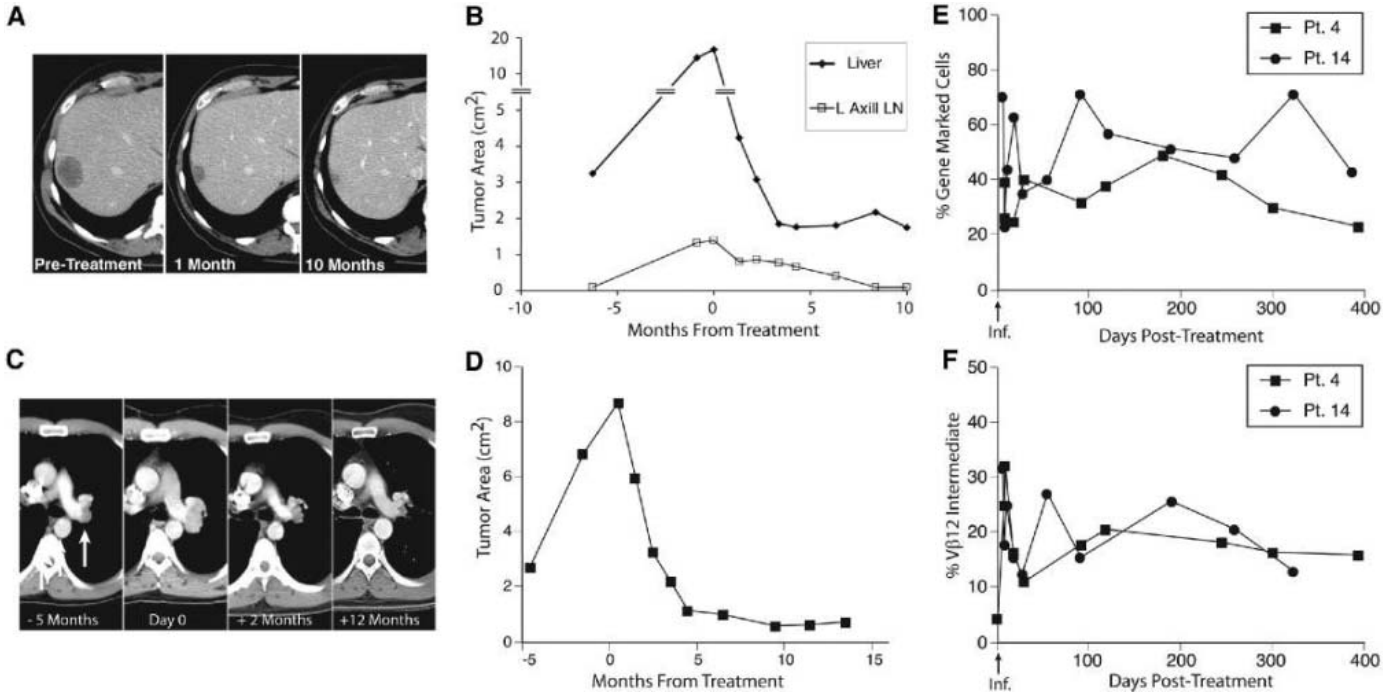


Fig. 3. Cancer regression in two patients. (**A**) Computed tomography (CT) images of liver metastasis in patient 4 taken at pretreatment, 1 month, and 10 months after treatment with TCR-engineered T cells. (**B**) Size of liver and axillary tumors and tempo of regression of tumor sites in patient 4. Day 0, beginning of treatment. L Axill LN, left axillary lymph node. (**C**) CT images of hilar lymph node metastasis in patient 14; pretreatment, day 0, and 2 months and 12 months after treatment. The white arrow indicates the mass in the lung hilum. (**D**) Size of tumor and tempo of regression in patient 14. (**E**) Quantitation of gene-marked cells in the PBMCs of patients 4 and 14 was determined by real-time quantitative PCR. Pt, patient. Day of infusion (Inf.) indicated by arrow. (**F**) The percentage of $CD8^+$/$V\beta12^+$ cells in the intermediate gate (*13*) in the circulation of patients 4 and 14.

to the lack of cell persistence seen in cohort 1 patients (Fig. 2A), patients in cohorts 2 and 3 (Fig. 2, B to D) all exhibited persistence of the transduced cells at >9% at 1 and 4 weeks after treatment (range, 9 to 56%). All eight patients who provided samples >50 days after treatment exhibited cell persistence of >17%, and this level of persistence was durable in seven patients during a >90-day monitoring period. In one patient (patient 14), >60% of circulating lymphocytes were positive for the gene-marked cells (Fig. 2C).

In 14 patient samples tested at one month after ACT, quantitative reverse transcription polymerase chain reaction (RT-PCR) assays revealed the presence of vector-derived RNA, confirming that gene expression continued (table S3). All but one of 15 patients analyzed had increased levels of $CD8^+/V\beta12$ cells at 1 week after treatment, and the levels of 11 of the 15 patients were higher at 1 month as compared to pretreatment levels (Fig. 2E). Of 13 patients that were examined, all had increased MART-1 tetramer-binding cells after treatment (Fig. 2F), and 11 of 14 had increased numbers of enzyme-linked immunosorbent spot–positive cells (table S4).

There was, however, a discordance between the mean persistence of transduced cells at 1 month in cohorts 2 and 3 measured by PCR (26%) as compared to the measurement of Vβ12-expressing cells (8.1%) and of MART-1 tetramer-binding cells (0.8%). This discordance may in part be due to mispairing of the introduced TCR chains with the endogenous chain, as well as to the different sensitivities of the assays. The reduced expression of the transgene in the persisting cells at ≥1 month may also be a function of the described decrease (*15*) in the transcription of retrovirally inserted trangenes and the decline in metabolic activity during the conversion of activated cells to memory cells. This decrease in expression of the retroviral transgene would be expected to affect the measurement of tetramers, which relies on the aggregation of multiple receptors, more heavily than the detection of Vβ12 cells directly by antibody staining.

Most important, two patients demonstrated a sustained objective regression of their metastatic melanoma assessed by standard criteria [response evaluation criteria in solid tumors (RECIST)] (*16*). Patient 4, a 52-year-old male, had previously received treatment with interferon-α (IFN-α), a lymph node dissection, an experimental vaccine, and high-dose IL-2. The patient then developed progressive disease in the liver (4.4- by 3.3-cm mass) and axilla (1.3- by 1.2-cm mass). After treatment with the ACT protocol described above, he experienced complete regression of the axillary mass and an 89% reduction of the liver mass (Fig. 3, A and B), at which time it was removed. He remains clinically disease-free at 21 months after treatment. Patient 14, a 30-year-old male, previously received treatment consisting of a lymph node dissection, IFN-α, and high-dose IL-2. He developed an enlarging 4.0- by 2.5-cm mass in the lung hilum. After ACT treatment, he underwent regression of the hilar mass and is now clinically disease-free 20 months later (Fig. 3, C and D). Thus, two patients with rapidly progressive metastatic melanoma showed full clinical regression of disease after the transfer of genetically engineered autologous PBLs.

In responding patients 4 and 14, the number of gene-marked cells in the circulation (assumed to be 1% of total body lymphocytes) increased by factors of 1400 and 30, respectively, as compared to the number of infusion cells. At 1 year after infusion, both responding patients had sustained high levels (between 20 and 70%) of circulating gene-transduced cells (Fig. 3E). This high level of gene-marked cells was confirmed in patient 4 by limiting dilution T cell cloning of circulating lymphocytes at 1 year after treatment, which revealed that 42% (33 out of 79) of T cell clones contained the transgene as assessed by the PCR assay. These two patients also displayed Vβ12 cells that were detectable by antibody staining between 12 and 16% for >300 days after treatment (Fig. 3F). Patients 4 and 14 were also two of four patients who had >1% of circulating tetramer positive cells detectable for >15 days after cell infusion (Fig. 2F), and these two patients demonstrated anti-TAA reactivity in ex vivo coculture assays (table S5). No toxicities in any patient were attributed to the gene-marked cells. Although the genetically modified transferred cells exhibited decreased expression of the transgene with time in vivo, the functional activity was apparently sustained at a level sufficient to mediate the tumor regression that was seen.

Approaches to increase the expression and function of the transgene are being studied, including the possible use of lentiviral vectors, the use of more powerful promoters specific to T cells, the use of higher-affinity TCRs that can mediate CD8 independent antitumor reactivity in CD4 cells, the further optimization of T cell transduction methods, and the production of higher titer clinical-grade viruses. Approaches to prevent chain mispairing may include modification of the TCR constant regions, the insertion of single-chain receptors (*17*), or the genetic modification of hematopoietic stem cells (*18*). Because tumor specificity can be conferred on bulk PBL populations with high efficiency, it may be possible to select subpopulations of PBLs that have distinct antitumor qualities. Further genetic modification of PBLs to insert cytokine or tissue-homing molecules may be beneficial. Mouse models predict that increased lymphodepletion, either by the addition of total body irradiation to the preparative regimen or by the administration of a vaccine containing the antigen recognized by the transduced TCR, can also enhance treatment effectiveness (*19*, *20*), and these modifications are currently being explored in clinical trials.

In human subjects, normal autologous T lymphocytes, transduced ex vivo with anti-TAA–TCR genes and reinfused in cancer patients, can persist and express the transgene for a prolonged time in vivo and mediate the durable regression of large established tumors. Although the response rate (2 out of 15 patients or 13%) seen in cohorts 2 and 3 is lower than that achieved by the infusion of autologous TILs (50%), this method has potential for use in patients for whom TILs are not available. Engineering PBLs to express high-affinity TCRs recognizing the NY-ESO-1 or p53 antigens (Fig. 1A and table S1) enables the in vitro recognition of TAAs expressed on a variety of common cancers, and the use of these genetically engineered cells for the treatment of patients with common epithelial cancers deserves evaluation.

References and Notes

1. S. A. Rosenberg, *Immunity* **10**, 281 (1999).
2. J. N. Blattman, P. D. Greenberg, *Science* **305**, 200 (2004).
3. S. A. Rosenberg, P. Spiess, R. Lafreniere, *Science* **233**, 1318 (1986).
4. M. E. Dudley *et al.*, *J. Clin. Oncol.* **23**, 2346 (2005).
5. M. E. Dudley *et al.*, *Science* **298**, 850 (2002).
6. M. Krogsgaard, M. M. Davis, *Nat. Immunol.* **6**, 239 (2005).
7. T. N. Schumacher, *Nat. Rev. Immunol.* **2**, 512 (2002).
8. M. Sadelain, I. Riviere, R. Brentjens, *Nat. Rev. Cancer* **3**, 35 (2003).
9. Y. Zhao *et al.*, *J. Immunol.* **174**, 4415 (2005).
10. R. A. Morgan *et al.*, *J. Immunol.* **171**, 3287 (2003).
11. M. S. Hughes *et al.*, *Hum. Gene Ther.* **16**, 457 (2005).
12. C. J. Cohen *et al.*, *J. Immunol.* **175**, 5799 (2005).
13. Materials and methods are available as supporting material on *Science* Online.
14. S. R. Riddell, P. D. Greenberg, *J. Immunol. Methods* **128**, 189 (1990).
15. D. B. Kohn *et al.*, *Nat. Med.* **4**, 775 (1998).
16. P. Therasse *et al.*, *J. Natl. Cancer Inst.* **92**, 205 (2000).
17. C. J. Cohen *et al.*, *Cancer Res.* **66**, 8878 (2006).
18. L. Yang, D. Baltimore, *Proc. Natl. Acad. Sci. U.S.A.* **102**, 4518 (2005).
19. L. Gattinoni *et al.*, *J. Exp. Med.* **202**, 907 (2005).
20. W. W. Overwijk *et al.*, *J. Exp. Med.* **198**, 569 (2003).

21. The authors acknowledge the expert help in the care of these patients provided by the Surgery Branch Immunotherapy Fellows; J. Gea-Banacloche for valuable advice concerning the management of infectious complications; the nurses on the 3NW and Surgical intensive care unit wards in the Clinical Center; and NIH, as well as A. Mixon and S. Farid for fluorescence-activated cell sorting analysis. We also thank K. Cornetta and the National Gene Vector Laboratory for production of the clinical-grade retroviral vector. This work was supported by the Intramural Research Program of the Center for Cancer Research, National Cancer Institute, NIH.

Supporting Online Material

www.sciencemag.org/cgi/content/full/1129003/DC1
Materials and Methods
Tables S1 to S5
References

20 April 2006; accepted 9 August 2006
Published online 31 August 2006;
10.1126/science.1129003
Include this information when citing this paper.

Ubiquitinated TDP-43 in Frontotemporal Lobar Degeneration and Amyotrophic Lateral Sclerosis

Manuela Neumann,[1,11]* Deepak M. Sampathu,[1]* Linda K. Kwong,[1]* Adam C. Truax,[1] Matthew C. Micsenyi,[1] Thomas T. Chou,[2] Jennifer Bruce,[1] Theresa Schuck,[1] Murray Grossman,[3,4] Christopher M. Clark,[3,4] Leo F. McCluskey,[3] Bruce L. Miller,[6] Eliezer Masliah,[7] Ian R. Mackenzie,[8] Howard Feldman,[9] Wolfgang Feiden,[10] Hans A. Kretzschmar,[11] John Q. Trojanowski,[1,4,5] Virginia M.-Y. Lee[1,4,5]†

Ubiquitin-positive, tau- and α-synuclein–negative inclusions are hallmarks of frontotemporal lobar degeneration with ubiquitin-positive inclusions and amyotrophic lateral sclerosis. Although the identity of the ubiquitinated protein specific to either disorder was unknown, we showed that TDP-43 is the major disease protein in both disorders. Pathologic TDP-43 was hyperphosphorylated, ubiquitinated, and cleaved to generate C-terminal fragments and was recovered only from affected central nervous system regions, including hippocampus, neocortex, and spinal cord. TDP-43 represents the common pathologic substrate linking these neurodegenerative disorders.

Ubiquitination of misfolded proteins that aggregate in the cytoplasm and/or nucleus of central nervous system (CNS) neurons is a key characteristic of neurodegenerative diseases (*1*). Misfolded disease proteins have been identified in many neurodegenerative disorders, but the identities of the ubiquitinated disease protein(s) in UBIs (defined here as ubiquitinated cytoplasmic, nuclear, and neuritic inclusions) in frontotemporal lobar degeneration (FTLD-U), the most common frontotemporal dementia (FTD) (*2*–*5*), and amyotrophic lateral sclerosis (ALS) are enigmatic. FTDs are clinically, genetically, and pathologically heterogeneous, but they are the most common cause of dementia under age 65 after Alzheimer's disease (AD) (*6*, *7*). FTDs present with progressive changes in social, behavioral, and/or language dysfunction (*7*–*9*), and some patients also develop parkinsonism or motor neuron disease (MND) (*2*, *10*). Conversely, ALS, a form of MND, is often associated with FTD (*10*), with UBIs identical to FTLD-U (*6*). Thus, clinical and pathological overlap in FTLD-U and ALS suggest they represent different manifestations of the same neurodegenerative disorder.

More than 30% of FTDs are familial, and many kindreds show linkage to chromosome 17 (*6*, *11*, *12*). However, FTD with parkinsonism linked to chromosome 17 (FTDP-17) usually shows tau pathology caused by pathogenic mutations in the microtubule-associated protein tau gene (*MAPT*) (*13*, *14*), FTDP-17T, but several FTDP-17 families are characterized by UBIs (FTDP-17U) without *MAPT* mutations (*15*–*17*). Recently, mutations in the progranulin gene (*PGRN*) were shown to be pathogenic for FTDP-17U (*11*, *12*). Because PGRN is not incorporated into UBIs in FTDP-17U (*11*, *12*), the FTLD-U disease protein remains to be identified.

On the basis of immunohistochemistry with ubiquitin and novel monoclonal antibodies (mAbs), at least three FTLD-U subtypes (types

[1]Center for Neurodegenerative Disease Research, Department of Pathology and Laboratory Medicine, [2]Department of Pharmacology, [3]Department of Neurology, [4]Alzheimer's Disease Core Center, [5]Institute on Aging, University of Pennsylvania School of Medicine, Philadelphia, PA 19104, USA. [6]Department of Neurology, University of California at San Francisco, CA 94117, USA. [7]Department of Neurosciences, University of California San Diego, School of Medicine, La Jolla, CA 92093, USA. [8]Department of Pathology, [9]Division of Neurology, University of British Columbia, Vancouver, British Columbia V6T 2B5, Canada. [10]Institute for Neuropathology, University of the Saarland, 66421 Homburg, Germany. [11]Center for Neuropathology and Prion Research, Ludwig-Maximilians University, 81377 Munich, Germany.

*These authors contributed equally to this work.
†To whom correspondence should be addressed. E-mail: vmylee@mail.med.upenn.edu

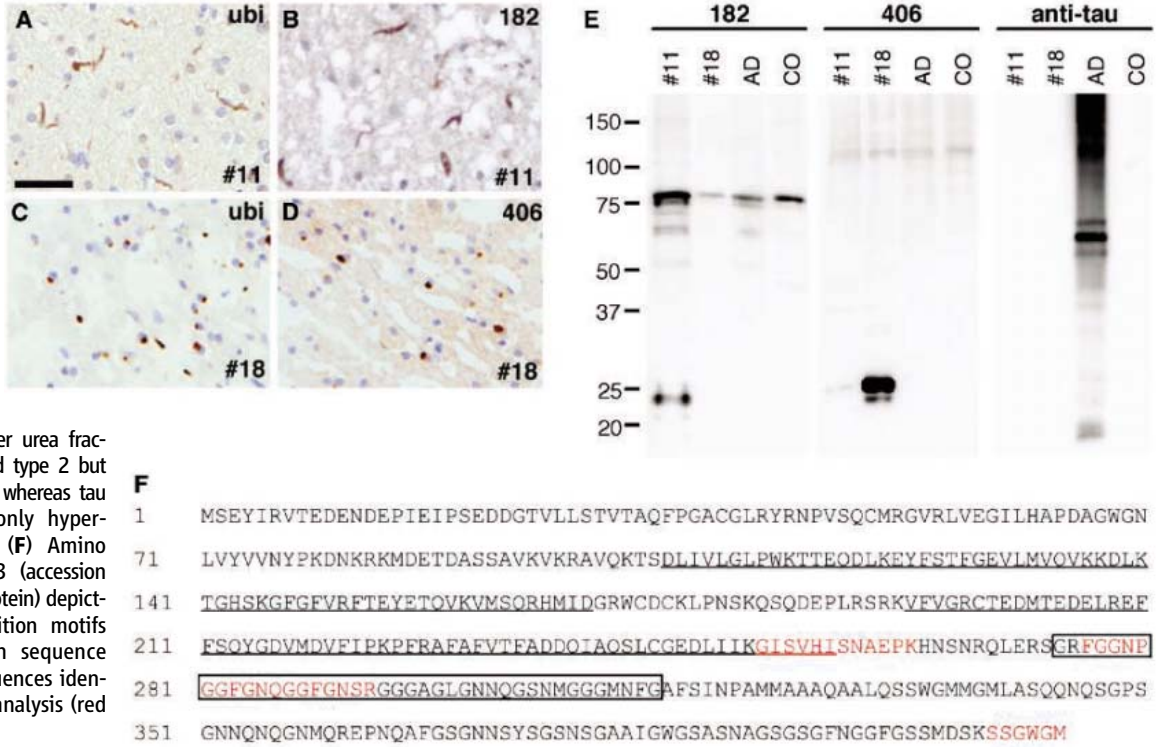


Fig. 1. Identification of TDP-43 as the major disease protein in UBIs of FTLD-U. (**A** to **D**) mAb 182 specifically labeled neuritic UBIs in FTLD-U type 1 [(A) and (B)], whereas mAb 406 immunostained UBIs in FTLD-U type 2 cases [(C) and (D)]. Scale bar in (A) corresponds to 25 μm for (A) to (D). (**E**) mAbs 182 and 406 detected disease-specific bands ~24 kD and ~26 kD, respectively, in frontal gray matter urea fractions of FTLD-U type 1 and type 2 but not in those of AD or CO, whereas tau mAbs T14/46 detected only hyperphosphorylated AD tau. (**F**) Amino acid sequence of TDP-43 (accession no. NP_031401, Entrez Protein) depicting the two RNA-recognition motifs (underlined), glycine-rich sequence (boxed), and peptide sequences identified through LC-MS/MS analysis (red highlights).

1 to 3) have been identified (*18*), suggesting that either different disease proteins or modifications of a single protein could underlie FTLD-U variants. Additional mAbs specific for distinct FTLD-U subtypes were generated (*19*), and mAb 182 was highly specific for UBIs in FTLD-U type 1 (Fig. 1, A and B, and table S1, nos. 1 to 12), whereas mAb 406 specifically labeled UBIs in FTLD-U type 2 cases (Fig. 1, C and D, and table S1, nos. 13 to 26). To further characterize the disease protein(s) recognized by mAbs 182 and 406, we performed immunoblots on urea fractions from FTLD-U types 1 and 2 brains. Notably, mAb 182 recognized an ~24-kD band in the urea fraction of type 1 that was not present in type 2, AD, or normal (CO) brains, whereas mAb 406 detected an ~26-kD band in FTLD-U type 2, but not in type 1, AD, or CO (Fig. 1E). As expected, tau antibodies detected insoluble pathological tau in AD but not in FTLD-U type 1, type 2, or CO (Fig. 1E), and mAbs 182 and 406 did not detect the 24- and 26-kD bands in FTLD-U type 3 and FTDP-17U cases.

To determine the identity of the 24- and 26-kD protein bands recognized by mAbs 182 and 406, respectively, we performed two-dimensional polyacrylamide gel electrophoresis (2D PAGE) immunoblots by using urea fractions from types 1 and 2 brains. MAbs 182 and 406 immunolabeled protein spots ~25 kD with a pI ~ 3.5 (fig. S1, A and C). The same spots were identified on duplicate Coomassie Blue–stained 2D PAGE gels (fig. S1, B and D) and analyzed by liquid chromatography–tandem mass spectrometry (LC-MS/MS). The same three peptides corresponding to amino acid residues 252 to 263, 276 to 293, and 409 to 414 of the TAR-DNA-binding protein 43 (TDP-43) were identified (Fig. 1F). Notably, the 409-414 peptide is at the extreme C terminus of TDP-43, suggesting that both 24- and 26-kD fragments are truncated in the middle of TDP-43 and extend to its C terminus.

The human gene encoding TDP-43 (*TARDP*) on chromosome 1 was cloned and shown to bind a polypyrimidine-rich motif in the HIV transactive response DNA (*20*), but it was later identified independently as part of a complex involved in the splicing of the cystic fibrosis transmembrane conductance regulator gene (*21*). TDP-43 contains two RNA-recognition motifs as well as a glycine-rich C-terminal sequence (*22*) (Fig. 1F) and is widely expressed in tissues, including heart, lung, liver, spleen, kidney, muscle, and brain (*21*). Because the same peptides were recovered from protein spots detected by mAbs 182 and 406, this suggests that both mAbs recognize specific conformations or posttranslational modifications of a C-terminal breakdown and/or cleavage product of TDP-43 unique to FTLD-U type 1 and 2, respectively.

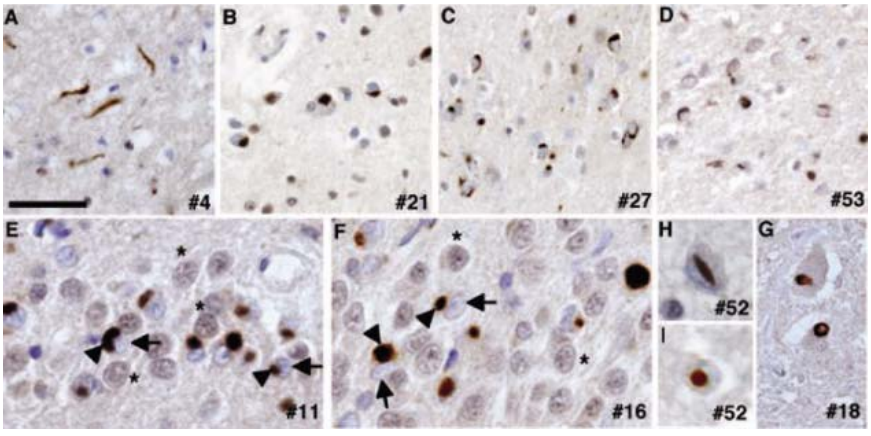


Fig. 2. Spectrum of FTLD-U neuropathology detected by anti–TDP-43. Immunohistochemistry of FTLD-U frontal cortex with anti–TDP-43 reveals robust staining of UBIs in FTLD-U (**A**) type 1, (**B**) type 2, (**C**) type 3, and (**D**) HDDD2. (**E** and **F**) Strong staining of UBIs (arrowheads) in hippocampal dentate granule neurons. Note clearing of nuclear TDP-43 (arrows) in UBI-bearing neurons compared that of with normal neurons (*). TDP-43–positive lentiform (**H**) and round (**G**) intranuclear UBIs in HDDD2 and Lewy body–like round inclusions in motor neurons of spinal cord (**I**). Scale bar in (A) corresponds to 50 μm [(A) to (D) and (G)], 25 μm [(E) and (F)] and 20 μm [(H) and (I)].

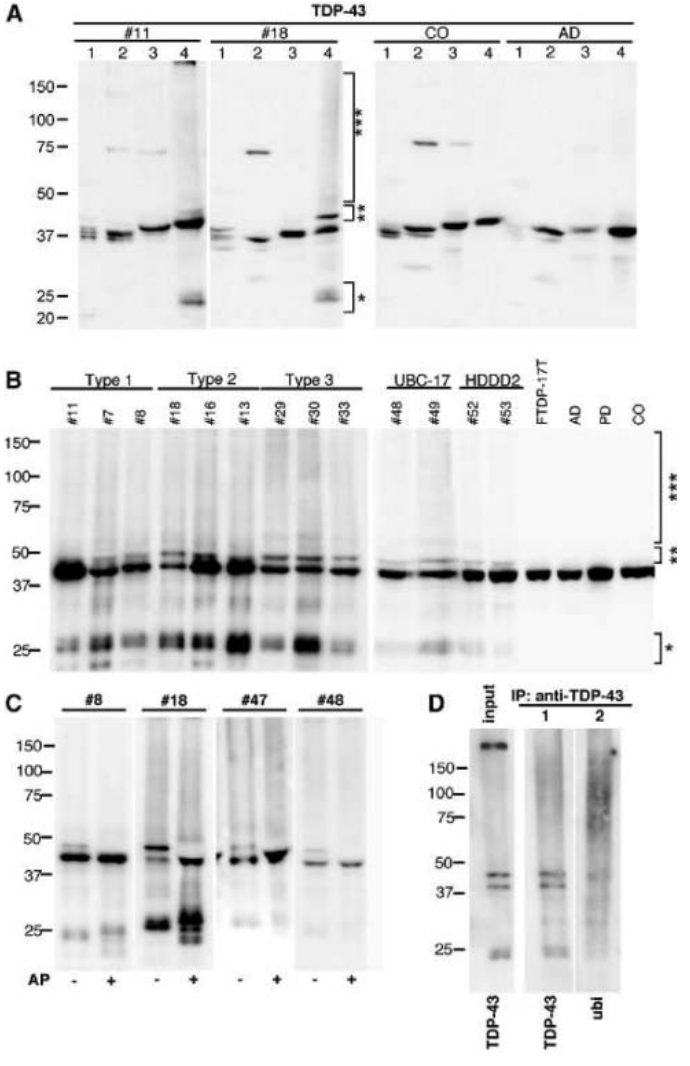


Fig. 3. Biochemical analyses of TDP-43 in sporadic and familial FTLD-U. (**A**) Immunoblots of sequential extracts from frontal cortex of FTLD-U types 1 and 2 with rabbit anti–TDP-43 showed pathologic ~25-kD bands (*), 45-kD bands (**), and high M_r smear (***) in the urea fraction. Lane 1, low salt; 2, high salt with triton x-100; 3, sarkosyl; 4, urea. (**B**) Immunoblots of urea fractions from hippocampal and temporal cortex of FTLD-U types 1 to 3 and frontal cortex of FTDP-17U showed the distinct pathological profile of TDP-43 not present in other neurodegenerative diseases or CO. (**C**) Dephosphorylation of FTLD-U urea extracts collapsed the 45-kD band into the 43-kD band and separated truncated TDP-43 into four immunoreactive ~23- to ~27-kD bands. (**D**) Immunoprecipitation of FTLD-U urea extract with anti–TDP-43 followed by immunoblotting with anti–TDP-43 (lane 2) and ubiquitin (Ub1B4) (lane 3) antibodies revealed that TDP-43 is ubiquitinated.

Double-labeling immunofluorescence studies showed that TDP-43 antibodies (anti–TDP-43) strongly immunolabeled UBIs detected by mAb 182 in FTLD-U type 1 cases and by mAb 406 in FTLD-U type 2 cases (fig. S2, A to F). Surprisingly, anti–TDP-43 robustly labeled UBIs that were not detected by mAbs 182 and 406 in FTLD-U type 3 cases, as well as UBIs in familial FTDP-17U brains (fig. S2, G to L). Anti–TDP-43 detected at least as many UBIs as ubiquitin antibodies or mAbs 182 and 406 in these FTLD-U brains.

Robust anti–TDP-43 staining was observed in affected cortical regions of FTLD-U type 1 (Fig. 2A and fig. S3, A and E), type 2 (Fig. 2B and fig. S3, B and F), and type 3 (Fig. 2C and fig. S3, C and G) cases, with the distinct morphology and distribution pattern characteristic of each of these FTLD-U subtypes (*18*). TDP-43–positive UBIs resembling those described for FTLD-U type 3 were detected in two separate FTDP-17U pedigrees [UBC-17 (*16*) and HDDD2 (*17*)] (Fig. 2D and fig. S3, D and H). Furthermore, strong TDP-43 staining was observed in UBIs of hippocampal dentate granule cells (Fig. 2, E and F) and in intranuclear UBIs characteristic of FTDP-17U cases (Fig. 2, G and H). Notably, TDP-43 was detectable in the nuclei of unaffected neurons but absent in nuclei of neurons with UBIs (asterisks, arrows, and arrowheads in Fig. 2, E and F), suggesting that TDP-43 redistributes from nucleus to cytoplasm in affected neurons. Furthermore, anti–TDP-43 labeled UBIs in the motor neurons of spinal cord and brain stem in FTLD-U cases with and without clinical signs of MND (Fig. 2I). Diagnostic inclusions of other neurodegenerative disorders were uniformly TDP-43–negative (fig. S3, I to T). Thus, TDP-43 is a highly specific disease protein found in neuronal UBIs of all FTLD-U subtypes and FTDP-17U.

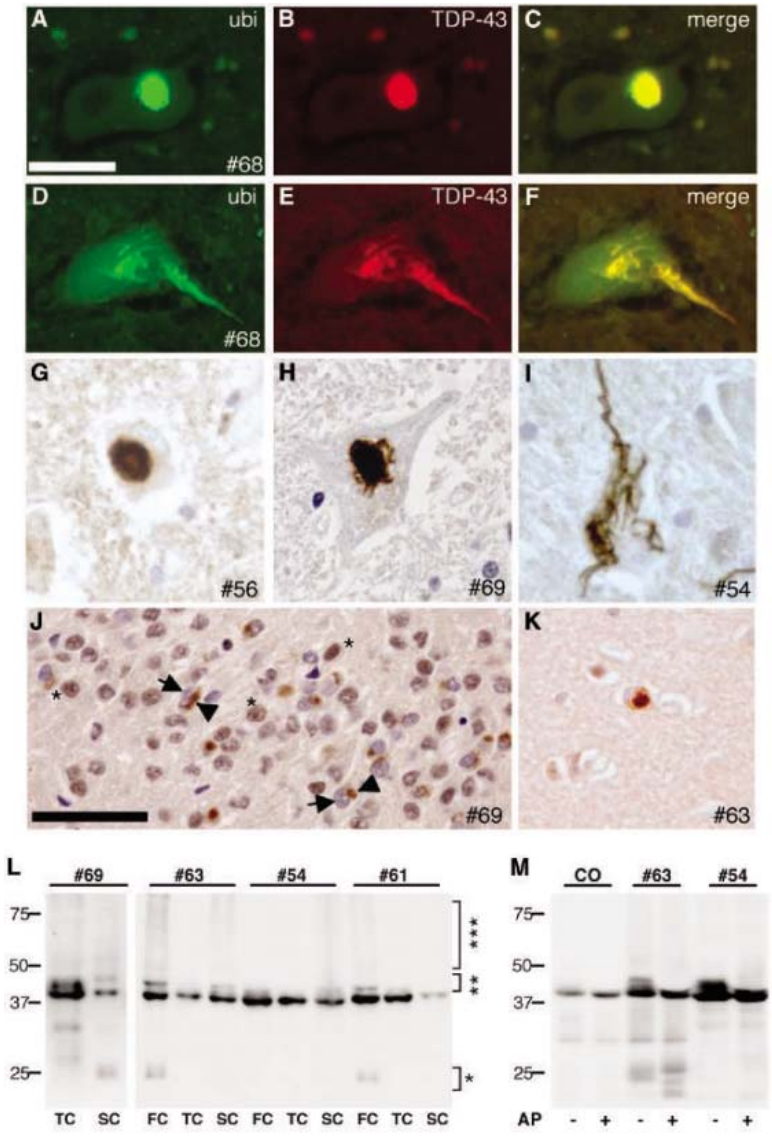


Fig. 4. UBIs in sporadic ALS were detected by anti–TDP-43. Double-label immunofluorescence with anti-ubiquitin (**A** and **D**) and anti–TDP-43 (**B** and **E**) showed colocalization in round (**C**) and skeinlike UBIs in spinal cord motor neuron (**F**). Merge images are shown in (C) and (F). Immunostaining with anti–TDP-43 labeled Lewy body–like (**G**), round (**H**), and skeinlike inclusions (**I**) in motor neurons of the spinal cord. Cytoplasmic UBIs in hippocampal dentate granule neurons (**J**) and few UBIs in frontal cortex (**K**) were also stained by TDP-43 (asterisk, normal nuclear staining; arrows, absence of nuclear staining in UBI-bearing neurons; arrowheads, UBIs). Scale bar in (A) corresponds to 25 μm [(A) to (I)]; scale bar in (J) corresponds to 50 μm [(J) and (K)]. (**L**) Immunoblots of urea fractions from frontal cortex (FC), temporal cortex (TC), and spinal cord (SC) of ALS cases probed with anti–TDP-43 demonstrated variable presence of the pathologic C-terminal fragments (*), 45-kD bands (**), and high M_r smear (***). (**M**) Immunoblots of dephosphorylated ALS urea extracts with anti–TDP-43 revealed collapse of the 45-kD band into the 43-kD band and increase in complexity of truncated TDP-43-immunoreactive bands ~23 to 27 kD.

To characterize TDP-43 protein biochemically, we sequentially extracted cortical gray matter from FTLD-U and FTDP-17U brains with buffers of increasing strength and analyzed the samples by immunoblot. Whereas full-length TDP-43 protein was present in all soluble and insoluble fractions of FTLD-U type 1 and type 2 as well as AD and CO, a strong labeling of ~25-kD bands similar to those detected by mAbs 182 and 406 was only detectable in the urea fractions of FTLD-U types 1 and 2, respectively (single asterisk in Fig. 3A). Further, a higher ~45-kD band and a high relative molecular mass (M_r) smear were recognized by anti–TDP-43 in the urea fractions of the FTLD-U brains compared with those of AD and CO (double and triple asterisks in Fig. 3A). Analyzing urea fractions extracted from hippocampus or frontal cortex of multiple FTLD-U brains demonstrates that this disease-specific TDP-43 signature was observed in all FTLD-U subtypes and familial FTDP-17U except in unaffected regions (e.g., cerebellum), and it was FTLD-U specific because it was not detected in CO or in other neurodegenerative disorders (e.g., AD and FTDP-17T) (Fig. 3B). Thus, the signature of pathological TDP-43 in FTLD-U includes the presence of C-terminal breakdown and/or cleavage products migrating at ~25 kD, a ~45-kD M_r variant, and a high-M_r TDP-43-immunoreactive smear, although quantities of pathological species of TDP-43 varied, possibly reflecting the extent of UBIs in diverse brain regions of different FTLD-U cases.

To determine what accounts for these disease-specific alterations of TDP-43 and because TDP-43 contains numerous potential phosphorylation sites, we next investigated the phosphorylation state of TDP-43 in FTLD-U. Dephosphorylation of urea fractions of FTLD-U brains showed collapsing of the 45-kD band into a 43-kD band (Fig. 3C) and separated the two C-terminal fragments into at least four distinct TDP-43 immunobands (Fig. 3C). Thus,

abnormal hyperphosphorylation of TDP-43 might play a role in FTLD-U pathogenesis. Because UBIs are defined by ubiquitin immunohistochemistry, we asked whether TDP-43 recovered from urea fractions of FTLD-U brains is ubiquitinated, and this was shown to be the case by immunoprecipitation studies using the rabbit polyclonal anti–TDP-43 followed by immunoblot analyses with both anti–TDP-43 and ubiquitin antibodies (Fig. 3D).

FTLD-U and ALS have been suggested to be part of a clinicopathological spectrum (*23*), sharing similar pathogenic mechanisms that affect different populations of CNS neurons. We examined classic ALS cases for the presence of TDP-43–positive UBIs (table S1, nos. 54 to 72). Although none of the inclusions typical of ALS were detected by mAbs 182 and 406, all UBIs (including skeinlike, round, and Lewy body–like inclusions) in motor neurons of ALS were robustly double-labeled by TDP-43 and ubiquitin antibodies (Fig. 4, A to F) and by single-label TDP-43 immunohistochemistry (Fig. 4, G to I). A significant number of ALS patients demonstrate UBIs in hippocampus and frontal and temporal cortex (*23*), which were also immunolabeled by TDP-43 (Fig. 4, J and K).

Immunoblots of urea fractions of spinal cord as well as frontal and temporal cortices of ALS cases demonstrated a disease-specific signature for TDP-43 similar to that described above for FTLD-U (Fig. 4L). Dephosphoryation of the urea fractions showed that the 45-kD band in ALS corresponds to pathologically hyperphosphorylated TDP-43 as in FTLD-U (Fig. 4M). However, because the presence of UBIs in ALS cases is more variable than their presence in FTLD-U, not all brain regions examined in all cases exhibited pathological TDP-43.

These studies identify TDP-43 as the major disease protein in the signature UBIs of FTLD-U and ALS. Although pathologically altered TDP-43 proteins were present in all sporadic and familial FTLD-U as well as ALS cases, there were subtle differences in these abnormal TDP-43 variants among the three FTLD-U subtypes, which may be the result of similar but not identical pathogenic mechanisms. The differential distribution of UBIs detected by ubiquitin antibodies in FTLD-U subtypes (*18*) supports this view.

TDP-43 is a ubiquitously expressed, highly conserved nuclear protein (*24*) that may be a transcription repressor and an activator of exon skipping (*21*, *25*, *26*) as well as a scaffold for nuclear bodies through interactions with survival motor neuron protein (*27*). TDP-43 is normally localized primarily to the nucleus, but our data indicate that, under pathological conditions in FTLD-U, TDP-43 is eliminated from nuclei of UBI-bearing neurons, a consequence of which may be a loss of TDP-43 nuclear functions. Moreover, nuclear UBIs are rare in sporadic FTLD-U because most pathological TDP-43 accumulates in neuronal cell bodies or their processes, and it is unclear whether physiological TDP-43 is present at significant quantities in the cytoplasm, axons, and dendrites of normal neurons. Lastly, both FTDP-17U pedigrees examined here contain *PGRN* gene mutations (*11*), but the relation between TDP-43 and *PGRN*, which encodes a secreted growth factor involved in the regulation of multiple processes in development, wound repair, and inflammation (*28*), remains unclear.

The identification of TDP-43 as the major component of UBIs specific to sporadic and familial FTLD-U as well as sporadic ALS resolves a long-standing enigma concerning the nature of the ubiquitinated disease protein in these disorders. Thus, these diseases may represent a spectrum of disorders that share similar pathological mechanisms, culminating in the progressive degeneration of different selectively vulnerable neurons. These insights into the molecular pathology of FTLD-U and ALS can accelerate efforts to develop better therapies for these disorders.

References and Notes

1. M. S. Forman, J. Q. Trojanowski, V. M.-Y. Lee, *Nat. Med.* **10**, 1055 (2004).
2. J. R. Hodges *et al.*, *Ann. Neurol.* **56**, 399 (2004).
3. A. M. Lipton, C. L. White III, E. H. Bigio, *Acta Neuropathol.* (*Berlin*) **108**, 379 (2004).
4. J. K. Johnson *et al.*, *Arch. Neurol.* **62**, 925 (2005).
5. J. Shi *et al.*, *Acta Neuropathol.* (*Berlin*) **110**, 501 (2005).
6. M. S. Forman *et al.*, *Ann. Neurol.* **59**, 952 (2006).
7. G. M. McKhann *et al.*, *Arch. Neurol.* **58**, 1803 (2001).
8. M. Grossman, *J. Int. Neuropsychol. Soc.* **8**, 566 (2002).
9. D. Neary *et al.*, *Neurology* **51**, 1546 (1998).
10. C. Lomen-Hoerth, T. Anderson, B. Miller, *Neurology* **59**, 1077 (2002).
11. M. Baker *et al.*, *Nature* **442**, 916 (2006).
12. M. Cruts *et al.*, *Nature* **442**, 920 (2006).
13. P. Poorkaj *et al.*, *Ann. Neurol.* **43**, 815 (1998).
14. M. Hutton *et al.*, *Nature* **393**, 702 (1998).
15. R. Rademakers *et al.*, *Mol. Psychiatry* **7**, 1064 (2002).
16. I. R. Mackenzie *et al.*, *Brain* **129**, 853 (2006).
17. C. L. Lendon *et al.*, *Neurology* **50**, 1546 (1998).
18. D. M. Sampathu *et al.*, *Am. J. Pathol.*, in press.
19. Materials and methods are available as supporting material on *Science* Online.
20. S. H. Ou, F. Wu, D. Harrich, L. F. Garcia-Martinez, R. B. Gaynor, *J. Virol.* **69**, 3584 (1995).
21. E. Buratti *et al.*, *EMBO J.* **20**, 1774 (2001).
22. H. Y. Wang, I. F. Wang, J. Bose, C. K. Shen, *Genomics* **83**, 130 (2004).
23. I. R. Mackenzie, H. H. Feldman, *J. Neuropathol. Exp. Neurol.* **64**, 730 (2005).
24. Y. M. Ayala *et al.*, *J. Mol. Biol.* **348**, 575 (2005).
25. P. A. Mercado, Y. M. Ayala, M. Romano, E. Buratti, F. E. Baralle, *Nucleic Acids Res.* **33**, 6000 (2005).
26. E. Buratti, A. Brindisi, F. Pagani, F. E. Baralle, *Am. J. Hum. Genet.* **74**, 1322 (2004).
27. I. F. Wang, N. M. Reddy, C. K. Shen, *Proc. Natl. Acad. Sci. U.S.A.* **99**, 13583 (2002).
28. Z. He, A. Bateman, *J. Mol. Med.* **1**, 600 (2003).

29. We thank the Penn Proteomics Core Facility for the LC-MS/MS and M. Forman for critical comments on the manuscript. This work was funded by NIH (grants AG10124, AG17586, and T32 AG00255) and German Brain Bank "Brain-Net" (grant 01GI0299).

Supporting Online Material

www.sciencemag.org/cgi/content/full/314/5796/130/DC1
Material and Methods
Figs. S1 to S3
Table S1
References

21 August 2006; accepted 8 September 2006
10.1126/science.1134108

Infectious Prions in the Saliva and Blood of Deer with Chronic Wasting Disease

Candace K. Mathiason,[1] Jenny G. Powers,[3] Sallie J. Dahmes,[4] David A. Osborn,[5] Karl V. Miller,[5] Robert J. Warren,[5] Gary L. Mason,[1] Sheila A. Hays,[1] Jeanette Hayes-Klug,[1] Davis M. Seelig,[1] Margaret A. Wild,[3] Lisa L. Wolfe,[6] Terry R. Spraker,[1,2] Michael W. Miller,[6] Christina J. Sigurdson,[1] Glenn C. Telling,[7] Edward A. Hoover[1]*

A critical concern in the transmission of prion diseases, including chronic wasting disease (CWD) of cervids, is the potential presence of prions in body fluids. To address this issue directly, we exposed cohorts of CWD-naïve deer to saliva, blood, or urine and feces from CWD-positive deer. We found infectious prions capable of transmitting CWD in saliva (by the oral route) and in blood (by transfusion). The results help to explain the facile transmission of CWD among cervids and prompt caution concerning contact with body fluids in prion infections.

The prion diseases, or transmissible spongiform encephalopathies (TSEs), are chronic, degenerative, neurological diseases with uniformly fatal outcomes. TSEs are characterized by the conversion of the normal cellular prion protein (PrP^{c}) to an aberrant insoluble partially protease-resistant isoform (PrP^{res}). CWD, a transmissible spongiform encephalopathy of cervids (deer, elk, and moose), was first observed in the 1960s in captive deer and free-ranging deer and elk in northeastern Colorado and southeastern

Wyoming (*1*–*4*). CWD has now been identified in 14 states in the United States and two Canadian provinces. Despite its facile transmission, the exact mode of CWD infection has not been determined. Indeed, surprisingly little is known about the transmission of naturally occurring TSEs. For example, scrapie in sheep has been recognized for centuries, yet the precise mode of natural transmission remains unclear (*5*, *6*).

To determine whether infectious prions capable of transmitting CWD are present in body fluids and excreta of CWD-infected deer (CWD+), we exposed four cohorts (numbered 1 to 4, $n = 3$ to 4 per cohort) of 6-month-old CWD-naïve hand-raised white-tailed deer (*Odocoileus virginianus*) fawns from Georgia, United States (Table 1) to blood, saliva, a combination of urine and feces, or brain from free-ranging or captive CWD+ mule deer (*Odocoileus hemionus*) from Colorado, United States (tables S1 and S2). A control cohort (cohort 5, $n = 2$) received matching inocula collected from confirmed CWD-negative white-tailed deer (*O. virginianus*) from Georgia, United States. Because polymorphism in the normal prion protein gene (PRNP) may influence CWD susceptibility or incubation time in white-tailed deer, PRNP codon 96 genotype for each deer was determined (table S2) (*7*).

The deer fawns were housed in separate isolation suites under strict isolation conditions to exclude adventitious sources of prion exposure [supporting online material (SOM) text], thus permitting conclusions based on only the point-source exposure. After inoculation, the deer were monitored for CWD infection by serial tonsil biopsy performed at 0, 3, 6, and 12 months postinoculation (pi), and at termination (18 to 22 months pi). Equal portions of tissue were collected and stored (–70°C or fixed in 10% formalin) at each serial collection time point (tonsil) and at study termination (palantine tonsil, brain, and retropharyngeal lymph nodes) for the detection of the protease-resistant abnormal prion protein associated with CWD (PrP^{CWD}) (*8*).

Serial tonsil biopsy of each recipient deer revealed that infectious CWD prions were present in saliva and blood from CWD+ donor deer (Table 2). As expected, PrP^{CWD} was demonstrated between 3 and 12 months pi in tonsil biopsies of all four animals inoculated either orally or intercranially with CWD+ brain (cohort 4). More notably, PrP^{CWD} was detected in tonsil biopsies of two of three deer each in both the saliva and blood cohorts (numbers 1 and 2) at 12 months pi. By contrast, deer in the urine and feces inoculation cohort 3 remained tonsil biopsy negative for PrP^{CWD} throughout the 18-month study. Animals in the negative control inoculation cohort 5 also remained tonsil biopsy negative throughout the study.

Deer cohorts 1 (blood), 2 (saliva), and 3 (urine and feces) were electively euthanized at 18 months pi to permit whole-body examination for PrP^{CWD}. The greatest scrutiny was directed toward those tissues previously established to have highest frequency of PrP^{CWD} deposition in infected deer and generally regarded as the most sensitive indicators of infection—medulla oblongata and other brainstem regions, tonsil, and retropharyngeal lymph node. We found unequivocal evidence of PrP^{CWD} in brain and lymphoid tissue of all six tonsil biopsy–positive deer in cohorts 1 (blood) and 2 (saliva), whereas all deer in cohorts 3 and 5 were negative for PrP^{CWD} in all tissues (Table 2 and Figs. 1 and 2).

The transmission of CWD by a single blood transfusion from two symptomatic and one asymptomatic CWD+ donor is important in at least three contexts: (i) It reinforces that no tissue from CWD-infected cervids can be considered free of prion infectivity; (ii) it poses the possibility of hematogenous spread of CWD, such as through insects; and (iii) it provides a basis for seeking in vitro assays sufficiently sensitive to demonstrate PrP^{CWD} or alternate prion protein conformers in blood—one of the grails of prion biology and epidemiology.

The identification of blood-borne prion transmission has been sought before with mixed results (*9*–*11*). Bovine spongiform encephalopathy and scrapie have been transmitted to naïve sheep through the transfer of 500 ml of blood or buffy coat white blood cells from infected sheep (*12*, *13*). In addition, limited but compelling evidence argues for the transmission of variant Creutzfeldt-Jakob disease (vCJD) through blood from asymptomatic donors (*14*–*16*). Even in sporadic CJD, PrP^{res} has been found in periph-

Table 1. CWD prion bioassay inoculation cohorts. Cohort 1 fawns received either a single intraperitoneal (IP) inoculation of 250 ml of frozen citrated blood ($n = 2$) or an intravenous (IV) transfusion with 250 ml fresh citrated whole blood ($n = 1$) each from a single CWD+ donor. Cohort 2 fawns received a total of 50 ml saliva, each from a different CWD+ donor, orally (PO) in three doses over a 3-day period. Cohort 3 fawns received a total of 50 ml urine and 50 g of feces PO, each from a different CWD+ donor, in divided doses over a 3- to 14-day period. As positive controls, cohort 4 fawns were inoculated with a 10% brain homogenate from a CWD+ donor deer through either a single intracranial (IC) injection of 1 g equivalent of brain ($n = 2$) or PO with a total of 10 g equivalents of brain ($n = 2$) divided over a 3-day period. Cohort 5 fawns ($n = 2$) were inoculated with equivalent amounts of each of the above materials from a single CWD-negative donor deer to serve as negative controls for the study.

Animal cohort	*n*	Inoculum	Route (*n*)	Amount	No. of inoculations
1	3	Blood	IV (1), IP (2)	250 ml	1
2	3	Saliva	PO (3)	50 ml	3
3	3	Urine and feces	PO (3)	50 ml + 50 g	3 to 14
4	4	Brain	IC (2), PO (2)	1 g (IC), 10 g (PO)	1 (IC), 3 (PO)
5	2	All of the above	PO (2)	All of the above	1 to 14

Table 2. PrP^{CWD} detection by longitudinal tonsil biopsy and necropsy of deer exposed to body fluids or excreta from CWD+ deer. PrP^{CWD} assay results for tonsil (T), brain (B) (medulla oblongata at obex), and retropharyngeal lymph node (RLN) are shown. The number of deer in which PrP^{CWD} was detected (*8*) is shown over the total number of deer in the cohort. One of the three original animals inoculated with urine and feces was euthanized prematurely 61 days pi due to a bacterial infection. The deer in cohorts 1, 2, and 3 were terminated at 18 months (mo.) pi. Two of the four cohort 4 deer were terminated at 20 and 21 months pi. The two cohort 5 deer were terminated at 22 months pi.

Animal cohort	Inoculum	Biopsy collection					
		3 mo. (T)	6 mo. (T)	12 mo. (T)	Termination		
					T	B	RLN
1	Blood	0/3	0/3	2/3	3/3	2/3	3/3
2	Saliva	0/3	0/3	2/3	3/3	2/3	3/3
3	Urine and feces	0/2	0/2	0/2	0/2	0/2	0/2
4	Brain	1/4	2/4	4/4	2/2	2/2	2/2
5	Negative samples	0/2	0/2	0/2	0/2	0/2	0/2

[1]Department of Microbiology, Immunology, and Pathology, [2]Veterinary Diagnostic Laboratory, College of Veterinary Medicine and Biological Sciences (CVMBS), Colorado State University (CSU), Fort Collins, CO 80523, USA. [3]Biological Resource Management Division, National Park Service, Fort Collins, CO 80525, USA. [4]Wildlife Artist Supply Company (WASCO) Inc., Monroe, GA 30655, USA. [5]Warnell School of Forestry and Natural Resources, University of Georgia, Athens, GA 30609, USA. [6]Wildlife Research Center, Colorado Division of Wildlife, Fort Collins, CO 80526, USA. [7]Department of Microbiology, Immunology and Molecular Genetics, University of Kentucky, Lexington, KY 40536, USA.

*To whom correspondence should be addressed. E-mail: edward.hoover@colostate.edu

eral organs of some patients (*17*). The present work helps establish that prion diseases can be transmitted through blood.

The presence of infectious CWD prions in saliva may explain the facile transmission of CWD. Cervid-to-cervid interactions (SOM text), especially in high density and captive situations, would be expected to facilitate salivary cross-contact (*11*, *18*, *19*). Salivary dissemination of prions may not be limited to CWD. Protease-resistant prion protein has been demonstrated in the oral mucosa, taste buds, lingual epithelium, vomeronasal organ, and olfactory mucosa of hamsters infected with transmissible mink encephalopathy (*19*) and ferrets infected with CWD (*20*). Although no instance of CWD transmission to humans has been detected, the present results emphasize the prudence of using impervious gloves during contact with saliva or blood of cervids that may be CWD-infected.

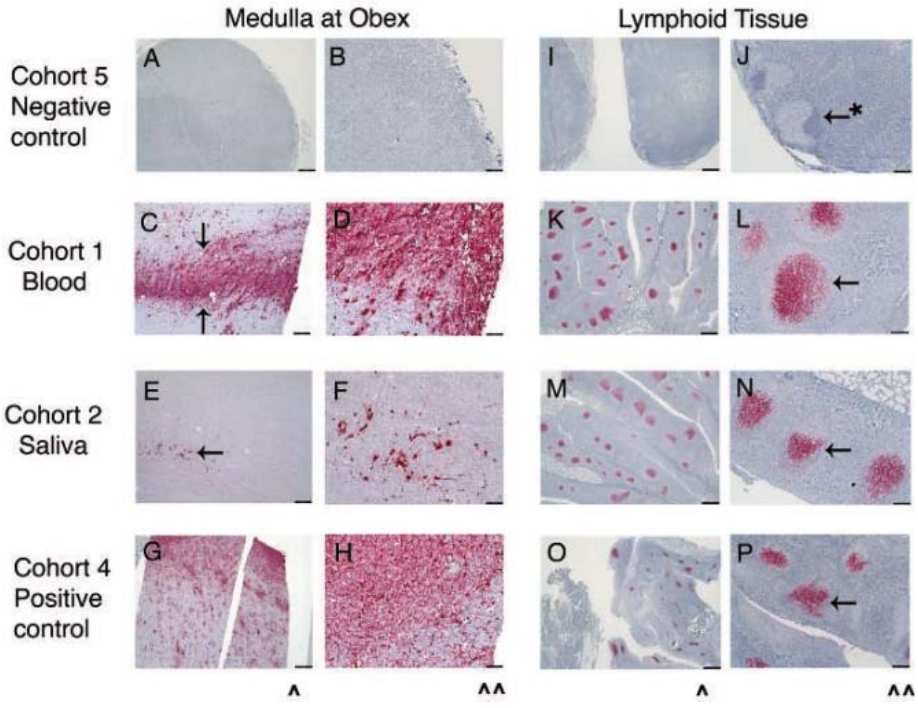


Fig. 1. PrP^{CWD} demonstrated by immunohistochemistry in tonsil, brain (medulla oblongata at obex), and retropharyngeal lymph node of deer receiving saliva or blood from CWD-infected donors. CWD immunohistochemistry is shown in the medulla at obex (**A** to **H**) and either tonsil or retropharyngeal lymph node (**I** to **P**) (*8*). Arrows indicate PrP^{CWD} staining (red) within brain and lymphoid follicles. Arrow with asterisk indicates lymphoid follicle negative for PrP^{CWD}. ^, scale bar = 550 μm; ^^, scale bar = 110 μm.

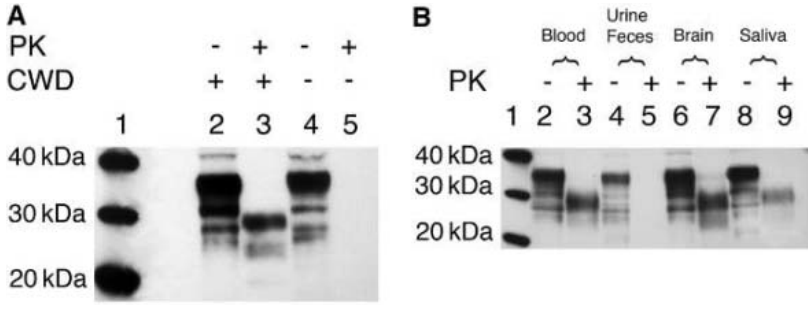


Fig. 2. Immunoblot demonstration of PrP^{CWD} in brain (medulla) of white-tailed deer. (**A**) PrP^{CWD} detection in positive and negative control deer (*8*). Lane 3 demonstrates the expected molecular weight shift upon partial proteinase K (PK) digestion of PrP^{CWD} in CWD+ deer, whereas lane 5 shows the complete digestion of PrP^{c} in CWD-negative deer. Molecular weight markers are indicated in lane 1. (**B**) Assay for PrP^{CWD} in medulla at obex homogenates for deer inoculated with blood, urine and feces, brain, and saliva, with and without PK digestion (*8*). Molecular weight markers are indicated in lane 1. Lanes 3, 7, and 9 demonstrate the detection of PrP^{CWD}, whereas lane 5 demonstrates the lack of PrP^{CWD}.

Environmental contamination by excreta from infected cervids has traditionally seemed the most plausible explanation for the dissemination of CWD (*21*). However, we could not detect PrP^{CWD} in cohort 3 deer inoculated repeatedly with urine and feces from CWD+ deer and examined up to 18 months pi (Table 2). There are several reasons to view this negative finding cautiously, including small sample size, elective preclinical termination, and potential variation in individual susceptibility that may be associated with the 96 G/S polymorphism in the PRNP gene (*7*, *22*). Although no genotype of white-tailed deer is resistant to CWD infection, PRNP genotypes S/S or G/S at codon 96 appear to have reduced susceptibility manifest by longer survival (*7*). Both deer in cohort 3 (urine and feces) were subsequently shown to be of the PRNP 96 G/S genotype. Thus, it is possible, although we think unlikely, that these deer had a prolonged incubation period (>18 months pi) before the amplification of PrP^{CWD} became detectable in tissues. Recent studies have shown that PrP^{res} is poorly preserved after incubation with intestinal or fecal content (*23*, *24*). Further research using cervid and surrogate cervid PrP transgenic mice (*25*) are indicated to continue to address the presence of infectious CWD prions in excreta of CWD+ deer and to provide a more substantial basis for reconsideration of the assumption that excreta are the chief vehicle for CWD dissemination and transmission.

The results reported here provide a plausible basis for the efficient transmission of CWD in nature. We demonstrate that blood and saliva in particular are able to transmit CWD to naïve deer and produce incubation periods consistent with those observed in naturally acquired infections (*3*, *26*). The time from exposure to first detection of PrP^{CWD} by tonsil biopsy was variable—as short as 3 months but as long as 18 months (likely underestimates due to sampling frequency). The results also reinforce a cautious view of the exposure risk presented by body fluids, excreta, and all tissues from CWD+ cervids.

References and Notes

1. E. S. Williams, S. Young, *J. Wildl. Dis.* **16**, 89 (1980).
2. E. S. Williams, S. Young, *J. Wildl. Dis.* **18**, 465 (1982).
3. E. S. Williams, S. Young, *Rev. Sci. Tech.* **11**, 551 (1992).
4. T. R. Spraker *et al.*, *J. Wildl. Dis.* **33**, 1 (1997).
5. R. T. Johnson, *Lancet Neurol.* **4**, 635 (2005).
6. W. J. Hadlow, R. C. Kennedy, R. E. Race, *J. Infect. Dis.* **146**, 657 (1982).
7. K. I. O'Rourke *et al.*, *J. Gen. Virol.* **85**, 1339 (2004).
8. Materials and methods are available as supporting material on *Science* Online.
9. C. M. Eklund, R. C. Kennedy, W. J. Hadlow, *J. Infect. Dis.* **117**, 15 (1967).
10. M. C. Clarke, D. A. Haig, *Vet. Rec.* **80**, 504 (1967).
11. W. J. Hadlow *et al.*, *J. Infect. Dis.* **129**, 559 (1974).
12. F. Houston, J. D. Foster, A. Chong, N. Hunter, C. J. Bostock, *Lancet* **356**, 999 (2000).
13. N. Hunter *et al.*, *J. Gen. Virol.* **83**, 2897 (2002).
14. L. Cervenakova *et al.*, *Transfusion* **43**, 1687 (2003).
15. C. A. Llewelyn *et al.*, *Lancet* **363**, 417 (2004).

16. A. H. Peden, M. W. Head, D. L. Ritchie, J. E. Bell, J. W. Ironside, *Lancet* **364**, 527 (2004).
17. M. Glatzel, E. Abela, M. Maissen, A. Aguzzi, *N. Engl. J. Med.* **349**, 1812 (2003).
18. M. W. Miller, E. S. Williams, *Curr. Top. Microbiol. Immunol.* **284**, 193 (2004).
19. C. DeJoia, B. Moreaux, K. O'Connell, R. A. Bessen, *J. Virol.* **80**, 4546 (2006).
20. M. P. Perrott, paper presented at the Molecular Mechanisms of Transmissible Spongiform Encephalopathies (Prion Diseases) Keystone Meeting, Snowbird, UT, 11 to 15 January 2005.
21. M. W. Miller, E. S. Williams, N. T. Hobbs, L. L. Wolfe, *Emerg. Infect. Dis.* **10**, 1003 (2004).
22. S. Supattapone *et al.*, *J. Virol.* **75**, 1408 (2001).
23. M. Jeffrey *et al.*, *J. Pathol.* **209**, 4 (2006).
24. C. Scherbel *et al.*, *Vet. Res.* **37**, 695 (2006).
25. S. R. Browning *et al.*, *J. Virol.* **78**, 13345 (2004).
26. E. S. Williams, M. W. Miller, *Rev. Sci. Tech.* **21**, 305 (2002).
27. We thank J. Grassi for his gift of PrP monoclonal antibody; M. Perrott for his technical expertise with the NaPTA methodology; K. O'Rourke and L. Hamburg for performing the PRNP genotype analysis; and C. Tedford for his expertise in deer behavior, facility design, and transport. We thank the office of the CSU Vice President for Research (A. Frank and K. Delehoy) for their support in establishing the CWD deer isolation facility. Animals were housed and cared for in accordance to CSU Animal Care and Use Committee regulations. This work was supported by the NIH, National Institute of Allergy and Infectious Diseases, contract N01-AI-25491.

Supporting Online Material
www.sciencemag.org/cgi/content/full/314/5796/133/DC1
Material and Methods
SOM Text
Tables S1 and S2
References

18 July 2006; accepted 29 August 2006
10.1126/science.1132661

Modulation of Cell Adhesion and Motility in the Immune System by Myo1f

Sangwon V. Kim,[1]* Wajahat Z. Mehal,[1,3] Xuemei Dong,[6] Volkmar Heinrich,[7]† Marc Pypaert,[4,5] Ira Mellman,[4,5] Micah Dembo,[7] Mark S. Mooseker,[4,8] Dianqing Wu,[6]‡ Richard A. Flavell[1,2]§

Although class I myosins are known to play a wide range of roles, the physiological function of long-tailed class I myosins in vertebrates remains elusive. We demonstrated that one of these proteins, Myo1f, is expressed predominantly in the mammalian immune system. Cells from Myo1f-deficient mice exhibited abnormally increased adhesion and reduced motility, resulting from augmented exocytosis of β2 integrin–containing granules. Also, the cortical actin that co-localizes with Myo1f was reduced in Myo1f-deficient cells. In vivo, Myo1f-deficient mice showed increased susceptibility to infection by *Listeria monocytogenes* and an impaired neutrophil response. Thus, Myo1f directs immune cell motility and innate host defense against infection.

In both mouse and human genomes, 16 genes encode conventional class II muscle and nonmuscle myosins, with 25 "unconventional" myosin genes encoding 11 other classes (*1*). Natural mutations of various myosin genes result in an array of genetic disorders, including cardiomyopathies, deafness, blindness, glomerular nephritis, and neuropathies (*2*, *3*). The class I myosins are the largest group of unconventional myosins and are evolutionarily ancient, existing in a wide range of species from yeast to vertebrates (*1*, *4*). Mice and humans have a total of eight class I myosin heavy-chain genes, six of which encode short-tailed forms (Myo1a, b, c, d, g, and h) and two of which encode long-tailed (amoeboid) forms (Myo1e and f) (*1*). All class I myosins consist of an N-terminal motor domain, light-chain–binding IQ motifs, and a basic tail homology 1 (TH1) domain thought to affect interactions with membranes (*2*). The long-tailed class I myosins have an additional proline-rich TH2 domain and a TH3 domain containing a single Src homology 3 (SH3) domain (*2*).

The class I myosins in *Dictyostelium* and yeast are involved in migration, phagocytosis, endocytosis, and actin remodeling (*5*, *6*). Short-tailed class I myosins in vertebrates are involved in more specialized functions, such as the adaptation of hair cells in the ear (*7*) and the transport of vesicles and organelles (*8*, *9*), as well as the structural maintenance of the enterocyte microvilli (*10*). However, the function of long-tailed class I myosins in vertebrates is poorly characterized (*11*–*14*).

Myo1f was first identified in our screen for differentially expressed genes in subsets of murine lymphocytes. In contrast to previous data suggesting the widespread expression of Myo1f in tissues (*15*), our results, which we obtained using specific probes, showed that Myo1f is selectively expressed in the spleen, mesenteric lymph nodes, thymus, and lung (Fig. 1A). By comparison, specific detection of Myo1e showed a predominant expression pattern in the spleen and mesenteric lymph nodes and moderate expression in the lung, small intestine, and large intestine (Fig. 1B). Within the lymphoid tissues, natural killer (NK) cells, macrophages, and dendritic cells were found to express considerable levels of both Myo1f and Myo1e; neutrophils and B cells showed selective expression of Myo1f and Myo1e, respectively (Fig. 1, C and D).

To determine the function of Myo1f in the vertebrate immune system, we generated Myo1f gene–deficient mice. We focused on neutrophils because Myo1f was detected exclusively in neutrophils (Fig. 1, C and D). Immunoglobulin G (IgG)–mediated phagocytosis was similar between wild-type and knockout (KO) neutrophils (Fig. 1E). To evaluate the degree of pathogen killing that follows phagocytosis, we measured the production of reactive oxygen species. Again, no considerable difference was detected between wild-type and KO neutrophils (Fig. 1F). Thus, Myo1f is dispensable for both the phagocytosis of bacteria and their destruction.

Integrin-mediated adhesion to the vascular endothelium is crucial in the process of neutrophil migration to infected tissue, and the dominant integrins involved in this process belong to the β2 integrin (CD18) family (*16*). Myo1f-deficient neutrophils exhibited stronger adhesion to integrin ligands, including the intercellular adhesion molecule–1 (ICAM-1) (CD54) and fibronectin (Fig. 2, A and B). Activation of neutrophils by the proinflammatory cytokine tumor necrosis factor–α did not compensate for this difference, suggesting that increased adhesion did not result from changes in the activation status of Myo1f-deficient cells. Experiments with a blocking antibody showed that most of the adhesion was mediated by β2 integrin (Fig. 2, A and B). In addition, Myo1f affected only integrin-mediated adhesion, not integrin-independent adhesion to polylysine-coated substrate (Fig. 2C) (*17*). Spreading of Myo1f-deficient neutrophils on ICAM-1 was also increased as compared to that of wild-type neutrophils (Fig. 2D). Increased spreading was not due to a loss of cortical tension (fig. S2), which acts to maintain the round shape of the cells (Fig. 2E). In contrast, myosin I double mutants in *Dictyostelium* exhibit abnormalities

[1]Section of Immunobiology, [2]Howard Hughes Medical Institute, [3]Section of Digestive Diseases, [4]Department of Cell Biology, [5]Ludwig Institute for Cancer Research, Yale University School of Medicine, New Haven, CT 06520, USA. [6]Department of Genetics and Developmental Biology, University of Connecticut Health Center, Farmington, CT 06030, USA. [7]Department of Biomedical Engineering, Boston University, Boston, MA 02215, USA. [8]Departments of Molecular, Cellular and Developmental Biology, and Department of Pathology, Yale University, New Haven, CT 06511, USA.

*Present address: Immunology Program, Memorial Sloan-Kettering Cancer Center, New York, NY 10021, USA.
†Present address: Department of Biomedical Engineering, University of California Davis, Davis, CA 95616, USA.
‡Present address: Vascular Biology and Transplantation Program and Department of Pharmacology, Yale University School of Medicine, New Haven, CT 06520, USA.
§To whom correspondence should be addressed. E-mail: richard.flavell@yale.edu

in surface morphology resulting from an inability to maintain a sufficient level of resting cortical tension (*5*, *18*). Together, these results suggest that, in the absence of Myo1f, neutrophils are more adherent to β2 integrin ligand.

Under resting conditions, no considerable difference was seen in the level of β2 integrin on the surface of Myo1f-deficient cells (Fig. 3A). In neutrophils, a large pool of integrin $\alpha_M\beta2$ (CD11b/CD18) is stored in cytoplasmic granules. This allows rapid up-regulation of integrin on the surface by granule exocytosis after stimulation (*19*). Myo1f-deficient neutrophils exhibited hyperinduction of cell-surface β2 integrin upon stimulation with integrin ligands (ICAM-1 and fibronectin) (Fig. 3A), although total cell levels of β2 integrin expression were comparable in wild-type and Myo1f-deficient cells (fig. S3). In addition, this up-regulation was specific for proteins in granules, because no difference was observed in the surface expression of the neutrophil marker Gr-1, which is not known to associate with neutrophil granules (fig. S4). The release of lactoferrin, a marker for the specific granules in which a large portion of integrin $\alpha_M\beta2$ is stored (*19*), is also significantly augmented in Myo1f-deficient cells upon stimulation with ICAM-1 and fibronectin (Fig. 3B). Thus, in the absence of Myo1f, increased granule exocytosis induced by integrin ligands leads to augmented β2 integrin on the cell surface, making neutrophils more adherent.

To examine the migratory properties of neutrophils, *N*-formyl-Met-Leu-Phe (*f*MLP)–gradient chemotaxis was measured by time-lapse microscopy. When observed on polylysine-coated glass, migration rates and efficiencies of wild-type and Myo1f-deficient neutrophils were similar (Fig. 3, C and E; fig. S5; and movies S1 and S2), in contrast to *Dictyostelium* myosin I mutants, which displayed low migration efficiency because of frequent turning (*20*). However, the migration rate of Myo1f-deficient neutrophils was severely impaired on fibronectin (Fig. 3D), with most neutrophils remaining attached on fibronectin (Fig. 3F and movies S3 and S4). These results suggest that, although the machinery for migration is intact in the absence of Myo1f, the increased integrin level on the surface makes these neutrophils more adherent and less motile (*21*).

To further explore the molecular basis of augmented granule exocytosis in Myo1f-deficient cells, we examined the intracellular location of Myo1f. Myo1f co-localizes with cortical actin filaments at the rim of the cells (Fig. 4A), and there is little co-localization with lactoferrin, which is a marker for neutrophil granule (fig. S6A). The low levels of diffuse staining in the cytoplasm are probably nonspecific, because this phenomenon is also observed on the KO neutrophils. Class I myosins in *Dictyostelium* and in yeast interact with the actin remodeling complex. Quantification of F-actin showed that there was a small but significant reduction in the amount of polymerized actin of resting neutrophils in the absence of Myo1f, whereas chemotaxis-related (*f*MLP-induced) actin polymerization was not affected (Fig. 4B), suggesting differential regulation of these actin structures (*22*). Cortical actin filaments near the plasma membrane, which constitute most of the F-actin in resting cells, can work as a barrier to granule secretion (*23*). Thus, it is possible that Myo1f indirectly inhibits granule exocytosis by modulating cortical actin. It is also possible that Myo1f exerts a direct opposing force by associating with granules. If so, this process would presumably occur as granules move into the cortex before exocytosis, because there was little co-localization of Myo1f with cytoplasmic granules even in cells that were stimulated for 7 min (fig. S6B). However, the cortical "rim" staining of Myo1f in activated cells is somewhat discontinuous and more diffuse, suggesting that there is some redistribution of Myo1f upon stimulation (fig. S6B).

Neutrophils play a crucial role in the early phase of protection against infection by *L. monocytogenes* (*24*). To test the effect of the phenotype described above in vivo during infection, wild-type and Myo1f-KO mice were challenged with *Listeria*. On day 2 after infection, Myo1f-KO mice contained 35 times more colony-forming units (CFUs) of *Listeria* than did wild-type littermate controls (Fig. 4C). In contrast to the well-formed microabscesses in wide-type mice (*25*) (Fig. 4D), poorly formed microabscesses in KO mice exposed large areas of infected hepatocytes (pale pink areas in Fig.

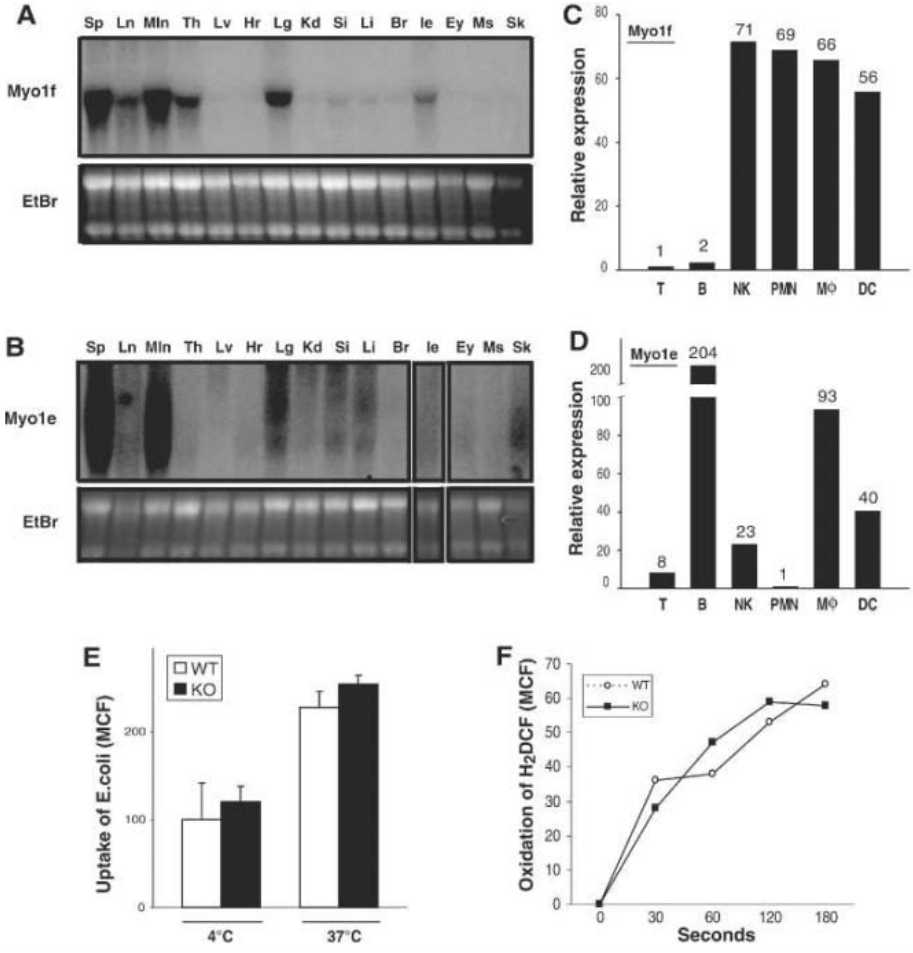


Fig. 1. Myo1f is expressed predominantly and differentially in the immune system. (**A** and **B**) mRNA levels of Myo1f (A) and Myo1e (B). EtBr, ethidium bromide. Sp, spleen; Ln, lymph node; Mln, mesenteric lymph node; Th, thymus; Lv, liver; Hr, heart; Lg, lung; Kd, kidney; Si, small intestine; Li, large intestine; Br, brain; Ie, inner ear; Ey, eye; Ms, muscle; Sk, skin. Relative mRNA levels of Myo1f (**C**) and Myo1e (**D**) in different cell types of the immune system determined by real-time reverse transcription polymerase chain reaction. T, T cells; B, B cells; NK, NK cells; PMN, neutrophils; MΦ, macrophages; DC, dendritic cells. Relative expression levels are displayed as a number on top of each bar. (**E**) IgG-mediated phagocytosis. Neutrophils were incubated with IgG-opsonized, fluorescein isothiocyanate (FITC)–labeled *Escherichia coli* at 37°C. Control neutrophils were incubated at 4°C. MCF, mean channel fluorescence; WT, wild type. Error bars represent SD of the mean. (**F**) The production of reactive oxygen species.

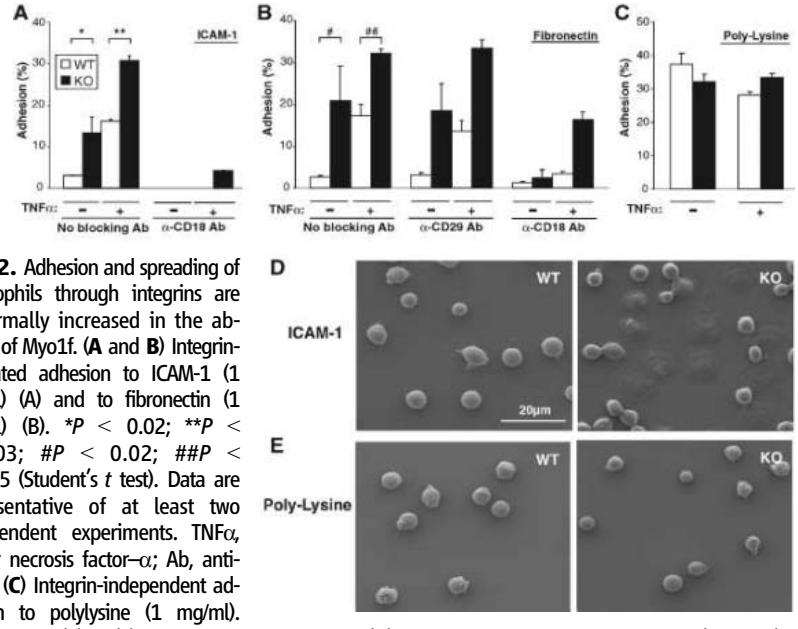


Fig. 2. Adhesion and spreading of neutrophils through integrins are abnormally increased in the absence of Myo1f. (**A** and **B**) Integrin-mediated adhesion to ICAM-1 (1 μg/ml) (A) and to fibronectin (1 μg/ml) (B). $*P < 0.02$; $**P < 0.0003$; $\#P < 0.02$; $\#\#P < 0.0005$ (Student's *t* test). Data are representative of at least two independent experiments. TNFα, tumor necrosis factor–α; Ab, antibody. (**C**) Integrin-independent adhesion to polylysine (1 mg/ml). Error bars in (A) to (C) represent SD of the mean. (**D**) Spreading of neutrophils to ICAM-1 (1 μg/ml), observed by SEM. (**E**) Surface morphology of neutrophils attached to polylysine (1 mg/ml), determined by SEM.

4E), indicating that the decreased ability to control *Listeria* is due to a defect in neutrophil mobilization. To further confirm that this in vivo defect is neutrophil-autonomous, we adoptively transferred wild-type and Myo1f-KO neutrophils into normal mice infected with *Listeria*. Myo1f-KO neutrophils were found in lower numbers in the recipient livers as compared to the observations in recipients of wild-type neutrophils (Fig. 4F), suggesting that the motility of neutrophils in vivo is compromised in the absence of Myo1f.

In accordance with the reported similarity between *Dictyostelium* and mammalian neutrophils (*26*), we observed that long-tailed class I myosin is conserved in cells from two different genera and supports cell motility. However, our results indicate that the ways in which myosin contributes to cell migration in the two models are quite different. Although long-tailed class I myosin in *Dictyostelium* prevents lateral pseudopod formation during migration (*20*), Myo1f in neutrophils inhibits exocytosis of integrin-containing granules, thereby preventing excess

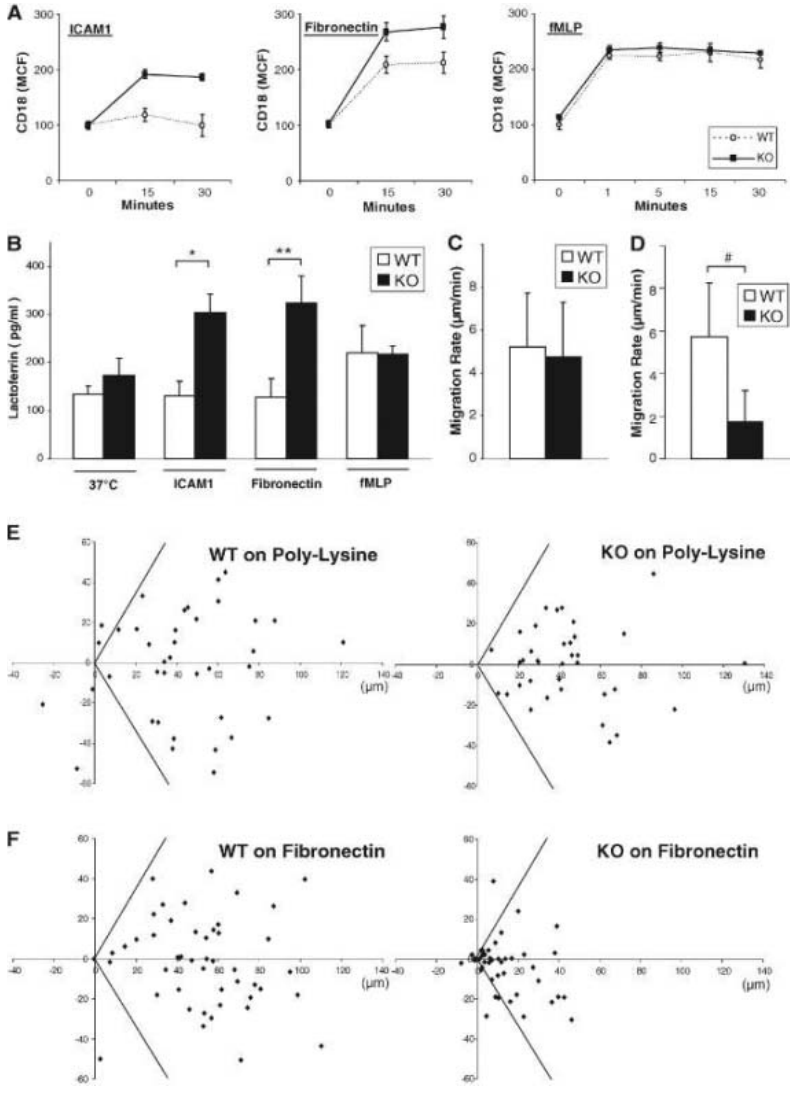


Fig. 3. In the absence of Myo1f, granule exocytosis is augmented, resulting in more β2 integrin on the surface and impaired migration on the extracellular matrix. (**A**) Neutrophils were stimulated with plate-bound ICAM-1 (1 μg/ml) or plate-bound fibronectin (1 μg/ml) or *f*MLP (10 μM) for the indicated times. Surface levels of β2 integrin (CD18) were represented by MCF, with fluorescence of WT neutrophils at 0 min given a value of 100 units. Data are representative of three independent experiments. (**B**) Secreted lactoferrin was measured in the supernatant at 30 min after stimulation. $*P < 5 \times 10^{-6}$; $**P < 5 \times 10^{-5}$ (Student's *t* test). Data are representative of two independent experiments. (**C** to **F**) Time-lapse video microscopy was used to examine neutrophil migration (movies S1 to S4) on polylysine (1 mg/ml) [(C) and (E)] or fibronectin (1 μg/ml) [(D) and (F)]. The relative position of each cell is shown as a diamond in the graphs [(E) and (F)] after 10 min of migration under a 10 μM *f*MLP gradient, assuming that the initial position of the cells was at (0, 0) in an *xy* plane. Lines with 120° of arc show the area facing the source of *f*MLP. Data are combined from six independent experiments. (C) and (D) Average migration rate of neutrophil on polylysine (C) and fibronectin (D). $\#P < 3 \times 10^{-14}$ (Student's *t* test). Error bars in (A) to (D) represent SD of the mean.

adhesion. Unlike protozoan cells, metazoan cells use integrins to interact with other cells and with the environment (*27*). Thus, our results suggest that, in higher organisms, long-tailed myosins have evolved to accommodate and make the best use of integrins to support cell motility. Our work also demonstrates that seemingly closely related myosin motors have different functions. Whereas short-tailed class I myosins facilitate vesicular transport (*8*, *9*), the long-tailed form Myo1f negatively regulates granule exocytosis in neutrophils. This work should lead to a better understanding of human immune defects that are currently of unknown origin and may provide an additional class of therapeutic targets for treating acute inflammation.

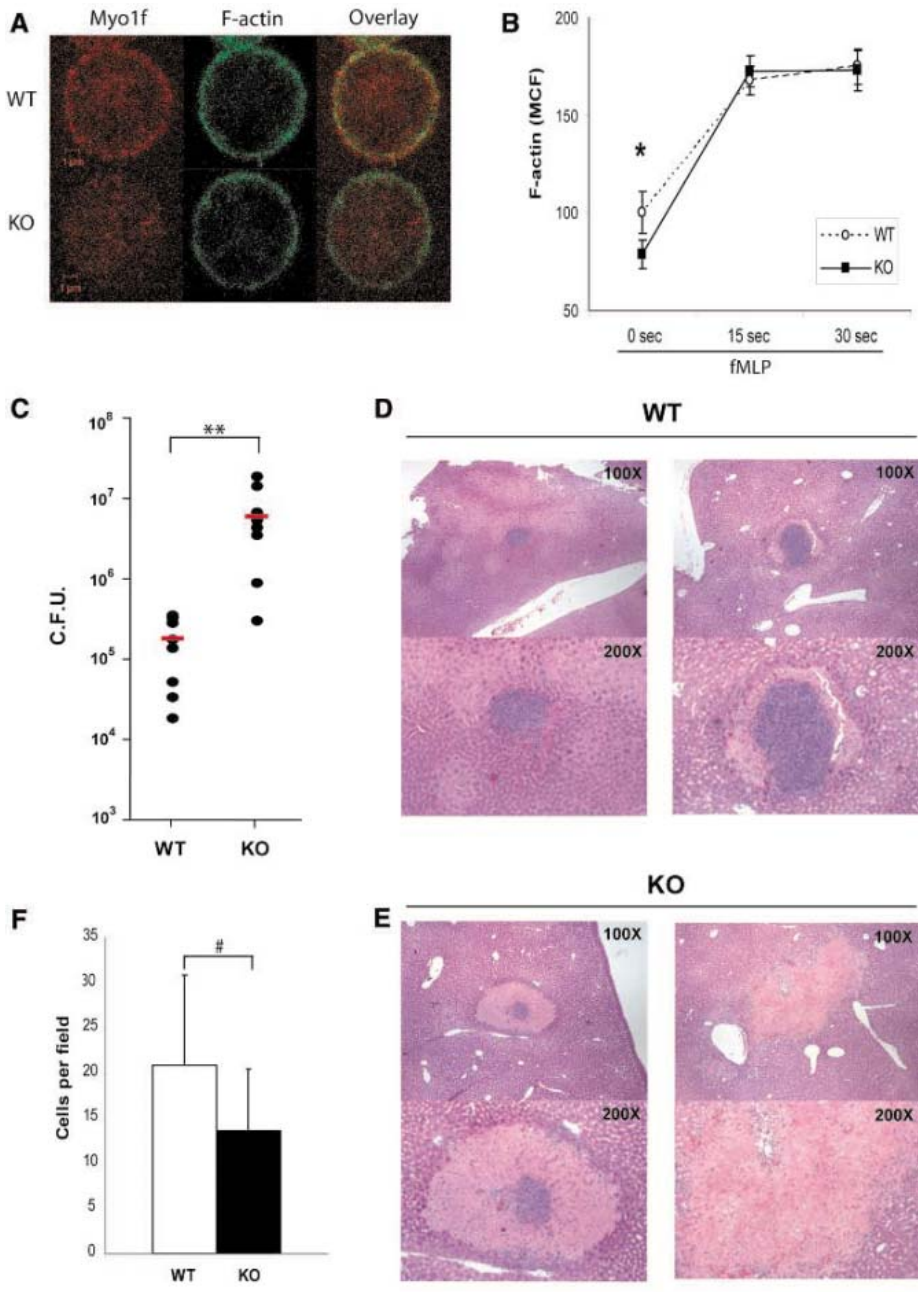


Fig. 4. Myo1f co-localizes with the cortical actin network, and neutrophil-dependent restriction of *L. monocytogenes* infection in vivo is impaired in the absence of Myo1f. (**A**) Confocal microscopy of neutrophils stained with antibody to Myo1f (red) and phalloidin for F-actin (green). (**B**) Quantification of F-actin in neutrophils after stimulation with 10 μM *f*MLP. MCF was used to quantify F-actin in neutrophils after staining with FITC-phalloidin, with fluorescence of WT neutrophils at 0 min given a value of 100 units. $*P < 6 \times 10^{-5}$ (Student's *t* test). (**C**) The number of *L. monocytogenes* in the spleen of WT ($n = 8$) and Myo1f KO ($n = 9$) mice observed 2 days after infection. $**P < 0.012$ (Student's *t* test). Red bars indicate mean values. (**D** and **E**) Microabscess formation in the liver of WT (D) and Myo1f KO (E) mice at 24 hours after infection. Cells with the strong blue color are neutrophils, and pale pink cells are infected hepatocytes undergoing apoptosis (*25*). (**F**) Adoptive transfer of neutrophils into mice infected with *Listeria*. The number of neutrophils localized in the liver was counted. $\#P < 5 \times 10^{-9}$ (Student's *t* test). Error bars in (B) and (F) represent SD of the mean.

References and Notes

1. J. S. Berg, B. C. Powell, R. E. Cheney, *Mol. Biol. Cell* **12**, 780 (2001).
2. M. Krendel, M. S. Mooseker, *Physiology* **20**, 239 (2005).
3. M. J. Redowicz, *Acta Biochim. Pol.* **49**, 789 (2002).
4. T. A. Richards, T. Cavalier-Smith, *Nature* **436**, 1113 (2005).
5. M. A. Titus, *J. Eukaryot. Microbiol.* **47**, 191 (2000).
6. A. E. Y. Engqvist-Goldstein, D. G. Drubin, *Annu. Rev. Cell Dev. Biol.* **19**, 287 (2003).
7. J. R. Holt *et al.*, *Cell* **108**, 371 (2002).
8. M.-N. Cordonnier, D. Dauzonne, D. Louvard, E. Coudrier, *Mol. Biol. Cell* **12**, 4013 (2001).
9. A. Bose *et al.*, *Nature* **420**, 821 (2002).
10. M. J. Tyska *et al.*, *Mol. Biol. Cell* **16**, 2443 (2005).
11. H. E. Stoffler, C. Ruppert, J. Reinhard, M. Bahler, *J. Cell Biol.* **129**, 819 (1995).
12. J. A. Swanson *et al.*, *J. Cell Sci.* **112**, 307 (1999).
13. A. H. Chen *et al.*, *Arch. Otolaryngol. Head Neck Surg.* **127**, 921 (2001).
14. T. Taki *et al.*, *Oncogene* **24**, 5191 (2005).
15. F. Crozet *et al.*, *Genomics* **40**, 332 (1997).
16. C. Wayne Smith, in *Clinical Immunology Principles and Practice*, R. R. Rich, T. T. Fleisher, W. T. Shearer, B. L. Kotzin, H. W. Schroeder, Eds. (Mosby, St. Louis, MO, 2001), pp. 2.22.21–15.
17. V. J. Thannickal *et al.*, *J. Biol. Chem.* **278**, 12384 (2003).
18. K. D. Novak, M. D. Peterson, M. C. Reedy, M. A. Titus, *J. Cell Biol.* **131**, 1205 (1995).
19. M. Faurschou, N. Borregaard, *Microbes Infect.* **5**, 1317 (2003).
20. D. Wessels, J. Murray, G. Jung, J. A. Hammer III, D. R. Soll, *Cell Motil. Cytoskeleton* **20**, 301 (1991).
21. S. P. Palecek, J. C. Loftus, M. H. Ginsberg, D. A. Lauffenburger, A. F. Horwitz, *Nature* **385**, 537 (1997).
22. S. L. Gupton *et al.*, *J. Cell Biol.* **168**, 619 (2005).
23. R. D. Burgoyne, A. Morgan, *Physiol. Rev.* **83**, 581 (2003).
24. J. W. Conlan, R. J. North, *J. Exp. Med.* **174**, 741 (1991).
25. H. W. Rogers, M. P. Callery, B. Deck, E. R. Unanue, *J. Immunol.* **156**, 679 (1996).
26. P. J. Van Haastert, P. N. Devreotes, *Nat. Rev. Mol. Cell Biol.* **5**, 626 (2004).
27. K. H. Martin, J. K. Slack, S. A. Boerner, C. C. Martin, J. T. Parsons, *Science* **296**, 1652 (2002).

28. The authors thank C. Marks in the Center for Cellular and Molecular Imaging at Yale University for help with scanning electron microscopy (SEM), L. Evangelisti and C. Hughes for technical assistance, L. Alexopoulou for technical advice, E. E. Eynon for a critical review of the manuscript, and F. Manzo for assistance with manuscript preparation. S.V.K. is a recipient of the Richard K. Gershon pre/postdoctoral fellowship. W.Z.M. and M.D. are recipients of grants from NIH (K08 DK02965 and R01 GM72002, respectively). M.S.M. is supported by grants from NIH (R01 DK25387 and R01 GM073823). R.A.F. is an Investigator of the Howard Hughes Medical Institute.

Supporting Online Material

www.sciencemag.org/cgi/content/full/314/5796/136/DC1
Materials and Methods
Figs. S1 to S6
References
Movies S1 to S4

29 June 2006; accepted 25 August 2006
10.1126/science.1131920

Genetic Variant BDNF (Val66Met) Polymorphism Alters Anxiety-Related Behavior

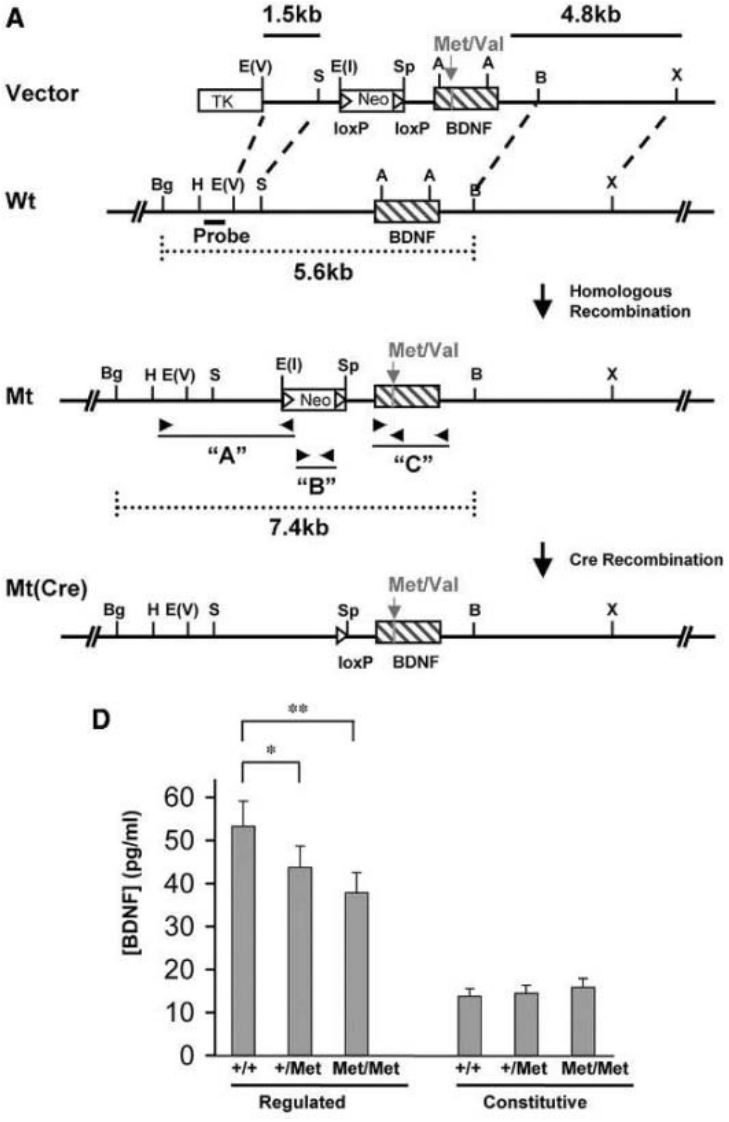
Zhe-Yu Chen,[1,4]† Deqiang Jing,[1*] Kevin G. Bath,[1*] Alessandro Ieraci,[1] Tanvir Khan,[1] Chia-Jen Siao,[2] Daniel G. Herrera,[1] Miklos Toth,[3] Chingwen Yang,[5] Bruce S. McEwen,[6] Barbara L. Hempstead,[2] Francis S. Lee[1,3]†

A common single-nucleotide polymorphism in the brain-derived neurotrophic factor (BDNF) gene, a methionine (Met) substitution for valine (Val) at codon 66 (Val66Met), is associated with alterations in brain anatomy and memory, but its relevance to clinical disorders is unclear. We generated a variant BDNF mouse ($BDNF^{Met/Met}$) that reproduces the phenotypic hallmarks in humans with the variant allele. $BDNF_{Met}$ was expressed in brain at normal levels, but its secretion from neurons was defective. When placed in stressful settings, $BDNF^{Met/Met}$ mice exhibited increased anxiety-related behaviors that were not normalized by the antidepressant, fluoxetine. A variant BDNF may thus play a key role in genetic predispositions to anxiety and depressive disorders.

Depression and anxiety disorders have genetic predispositions, yet the particular genes that contribute to this pathology are not known. One candidate gene is BDNF, because of its established roles in neuronal survival, differentiation, and synaptic plasticity. The recent discovery of a single-nucleotide polymorphism (SNP) in the *bdnf* gene (Val66Met), found only in humans, leading to a Met substitution for Val at codon 66 in the prodomain, has provided a valuable tool to assess potential contributions of BDNF to affective disorders. This polymorphism is common in human populations with an allele frequency of 20 to 30% in Caucasian populations (*1*). This alteration in a neurotrophin gene correlates with reproducible alterations in human carriers. Humans heterozygous for the Met allele have smaller hippocampal volumes (*2*–*4*)

[1]Department of Psychiatry, [2]Division of Hematology, Department of Medicine, [3]Department of Pharmacology, Weill Medical College of Cornell University, New York, NY 10021, USA. [4]School of Medicine, Shandong University, Jinan, Shandong 250012, People's Republic of China. [5]Gene Targeting Facility, The Rockefeller University, New York, NY 10021, USA. [6]Laboratory of Neuroendocrinology, The Rockefeller University, New York, NY 10021, USA.

*These authors contributed equally to this work
†To whom correspondence should be addressed. E-mail: zheyuchen@sdu.edu.cn (Z.-Y.C.); fslee@med.cornell.edu (F.S.L.)



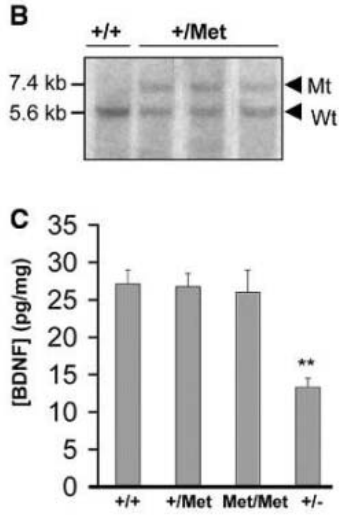


Fig. 1. Generation and validation of $BDNF_{Met}$ transgenic mice. (**A**) Schematic diagram of the strategy used to replace the coding region of the BDNF gene with $BDNF_{Met}$. The entire coding region is in exon V. For the variant BDNF, a point mutation has been made (G196A) to change the valine in position 66 to a methionine. (**B**) Southern blots of representative embryonic stem cell clones for $BDNF_{Met}$. Bgl II and Bam HI restriction enzyme digestion and 5′ external probe indicated in (A) were used to detect homologous replacement in the BDNF locus. The 5.6-kilobase (kb) WT and 7.4-kb rearranged variant DNA bands are indicated. (**C**) BDNF ELISA analyses of total BDNF levels from postnatal day 21 (P21) brain lysates from WT (+/+), heterozygous (+/Met), and homozygous (Met/Met) mice, as well as BDNF heterozygous KO mice (+/–) (**$P < 0.01$, Student's t test). (**D**) Hippocampal-cortical neurons obtained from embryonic day 18 (E18) $BDNF^{+/Met}$ (+/Met), $BDNF^{Met/Met}$ (Met/Met), and WT (+/+) pups were cultured. After 72 hours, media were collected under depolarization (regulated) or basal (constitutive) secretion conditions as described previously (*10*). Media were then concentrated and analyzed by BDNF ELISA. (*$P < 0.05$, **$P < 0.01$, Student's t test).

and perform poorly on hippocampal-dependent memory tasks (*5*, *6*). However, in genetic association studies for depression and anxiety disorders, there is little consensus as to whether this allele confers susceptibility.

The mechanisms that contribute to altered $BDNF_{Met}$ function have been studied in neuronal culture systems. The distribution of $BDNF_{Met}$ to neuronal dendrites and its activity-dependent secretion are decreased (*6*–*8*). These trafficking abnormalities are likely to reflect impaired binding of $BDNF_{Met}$ to a sorting protein, sortilin, which interacts with BDNF in the prodomain region that encompasses the Met substitution (*7*). However, fundamental questions remain as to how these in vitro effects relate to the in vivo consequences of this SNP in humans.

To generate a transgenic mouse in which $BDNF_{Met}$ is endogenously expressed, we designed a $BDNF_{Met}$ knock-in allele in which transcription of $BDNF_{Met}$ is regulated by endogenous BDNF promoters (Fig. 1, A and B). Heterozygous $BDNF^{+/Met}$ mice were intercrossed to yield $BDNF^{+/+}$, $BDNF^{+/Met}$, and $BDNF^{Met/Met}$ offspring at Mendelian rates. Brain lysates from $BDNF^{+/Met}$ and $BDNF^{Met/Met}$ mice showed comparable levels of BDNF as that of wild-type (WT) controls (Fig. 1C).

To assess whether there were global or selective defects in $BDNF_{Met}$ secretion, hippocampal-cortical neurons were obtained from $BDNF^{Met/Met}$, $BDNF^{+/Met}$, and WT embryos. Secretion studies were performed, and BDNF in the resultant media was measured by enzyme-linked immunosorbent assay (ELISA). There was no difference in constitutive secretion from either $BDNF^{+/Met}$ or $BDNF^{Met/Met}$ neurons (Fig. 1C). We observed a significant decrease in regulated secretion from both $BDNF^{+/Met}$ (18 ± 2% decrease, $P < 0.01$) and $BDNF^{Met/Met}$ (29 ± 3% decrease, $P < 0.01$) neurons (Fig. 1C). As the majority of BDNF is released from the regulated secretory pathway in neurons (*9*), impaired regulated secretion (29 ± 3%) from $BDNF^{Met/Met}$ neurons represents a significant decrease in available BDNF.

We first assessed an alteration associated with the Met allele in humans: decreased hippocampal volume (*3*, *4*, *10*). $BDNF_{Met}$ mice were histologically prepared for stereologic hippocampal volume estimation from Nissl-stained sections. Using Cavalieri volume estimation, we detected a significant decrease in hippocampal volume of 13.7 ± 0.7% and 14.4 ± 0.7% for $BDNF^{+/Met}$ or $BDNF^{Met/Met}$ mice, respectively, as compared with WT mice (Fig. 2A). This volume decrease was also comparable to the 13.8 ± 0.6% decrease in the heterozygous BDNF knock-out ($BDNF^{+/-}$) mice (Fig. 2A). We also measured striatal volume, because in human studies this structure has not been reported to be altered by the $BDNF_{Met}$ polymorphism (*2*, *3*), and we found no alteration in mouse striatal volumes across genotype (fig. S1).

Because secreted BDNF regulates neuronal differentiation, the decreased volume in $BDNF_{Met}$ mice may be accounted for by altered neuronal morphology. We used Golgi staining to visualize individual dentate gyrus neurons. At 8 weeks of age, there was no difference in cell soma area between $BDNF^{+/Met}$ and $BDNF^{Met/Met}$ mice and their WT controls (fig. S2). Next, we analyzed dendritic complexity in these same neurons (Fig. 2B). Sholl analysis revealed a decrease in dendritic arbor complexity at 90 μM and greater distances from the soma in $BDNF^{+/Met}$ and $BDNF^{Met/Met}$ mice (Fig. 2C). We also used fractal dimension analysis to quantify how com-

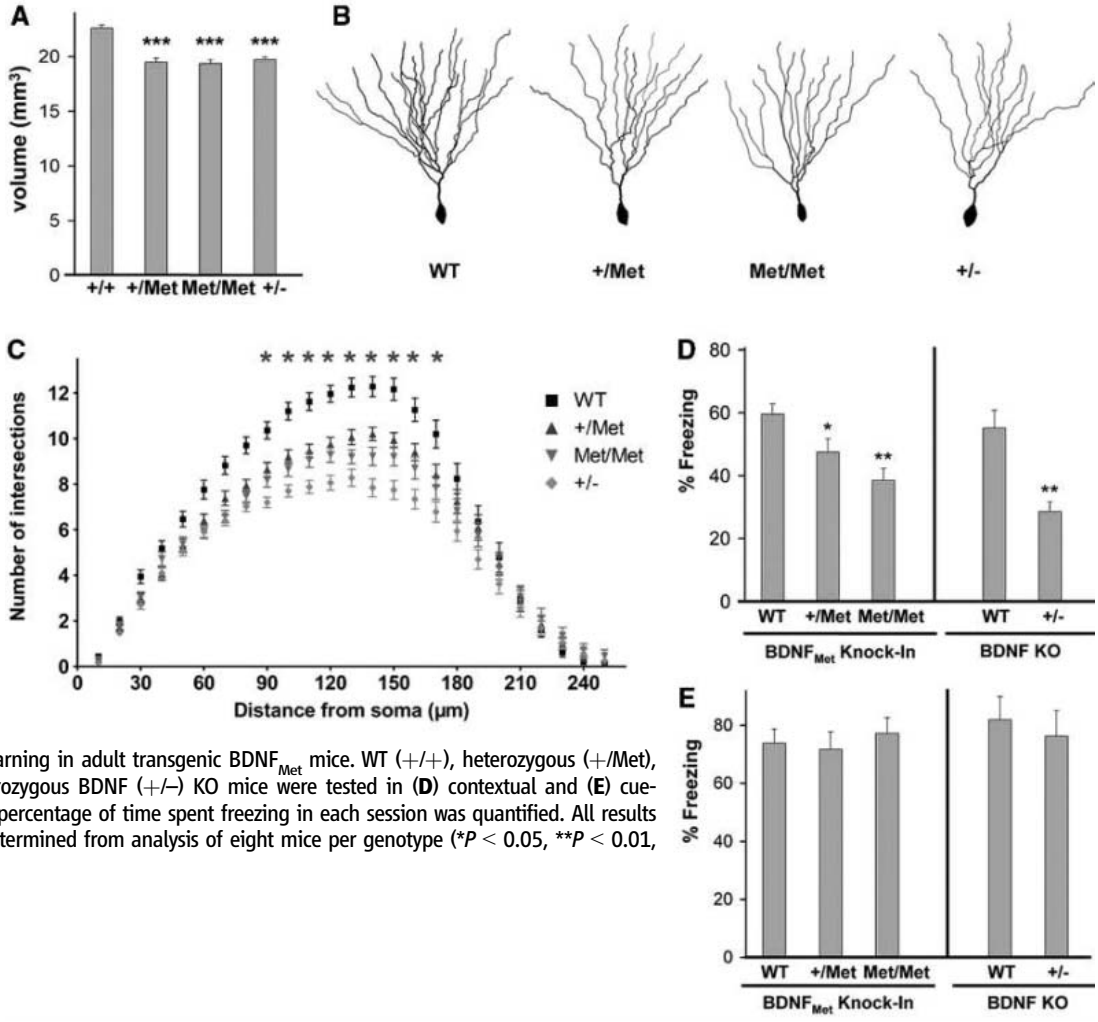


Fig. 2. Altered hippocampal anatomy and behavior in transgenic $BDNF_{Met}$ mice. (**A**) Total hippocampal volume estimations were obtained from Nissl-stained sections of adult (P60) hippocampi from WT (+/+), heterozygous (+/Met), homozygous (Met/Met), and heterozygous BDNF KO (+/–) mice by Cavalieri analyses. All results are presented as means ± SEM determined from analysis of six mice per genotype (***$P <$ 0.001, Student's t test). (**B**) Examples of Golgi-stained dentate gyrus neurons from P60 WT (+/+), heterozygous (+/Met), homozygous (Met/Met), and heterozygous BDNF KO (+/–) mice. (**C**) Sholl analyses of dentate gyrus neurons from P60 mice, five mice per genotype, 10 neurons per mouse. All results are presented as means ± SEM determined from analysis of five mice per genotype and statistics in comparison with WT controls (*$P <$ 0.001). Fear-conditioned learning in adult transgenic $BDNF_{Met}$ mice. WT (+/+), heterozygous (+/Met), homozygous (Met/Met), and heterozygous BDNF (+/–) KO mice were tested in (**D**) contextual and (**E**) cue-dependent fear conditioning. The percentage of time spent freezing in each session was quantified. All results are presented as means ± SEM determined from analysis of eight mice per genotype (*$P <$ 0.05, **$P <$ 0.01, Student's t test).

pletely a neuron fills its dendritic field (*11*). There was a significant decrease in dendritic complexity in dentate gyrus neurons from $BDNF^{+/Met}$ and $BDNF^{Met/Met}$ mice (fig. S3).

In humans, the other major alteration associated with the $BDNF_{Met}$ allele is impairment in hippocampus-dependent memory. We performed a test that selectively assesses hippocampus- and amygdala-dependent learning: fear conditioning. $BDNF^{+/Met}$ and $BDNF^{Met/Met}$ mice showed significantly less context-dependent memory than WT mice (Fig. 2D). In contrast, there was no difference in cue-dependent fear conditioning (Fig. 2E). Of note, prior studies of $BDNF^{+/-}$ mice also showed a deficit in contextual fear learning and no alteration in cue-dependent learning (*12*). The degree of memory impairment was related to the number of alleles of $BDNF_{Met}$ (Fig. 2D). $BDNF^{Met/Met}$ mice displayed other behavioral abnormalities similar to $BDNF^{+/-}$ mice (*15*), such as intermale aggressiveness (fig. S4). $BDNF^{Met/Met}$ mice also displayed elevated body weight, which was first evident at 2 months of age, similar to $BDNF^{+/-}$ mice (*15*) (fig. S5). Although other BDNF knockout (KO) mice with >50% decrease in BDNF levels previously exhibited increased locomotor activity (*13*, *14*), $BDNF^{+/-}$ (*15*), $BDNF^{+/Met}$, and $BDNF^{Met/Met}$ mice (figs. S6 and S7B) had no significant alterations in locomotor activity, which suggests a potential BDNF dose-related effect on activity.

BDNF has also been shown to regulate stress and anxiety-related behaviors. Acute or chronic stress leads to decreased BDNF expression in the hippocampus, with subsequent enhancement of anxiety-related behaviors (*16*). In addition, conditional BDNF KO mice exhibit enhanced avoidance of aversive settings (*13*). In this context, we focused on adult $BDNF_{Met}$ mice and performed two standard measures of anxiety-like behavior that place subjects in conflict situations. In comparison with littermate WT control mice, $BDNF^{Met/Met}$ mice had decreased exploratory behavior as demonstrated by a reduction in the percentage of time spent in the center compartment (Fig. 3A) and the number of entries into the center compartment (Fig. 3B) in the open-field test. $BDNF^{Met/Met}$ mice also exhibited, in the elevated plus maze test, a significant decrease in the percentage of time spent in open arms (Fig. 3C) and a significant reduction in the percentage of entries into open arms (Fig. 3D). In both tests, there were no significant differences in total distance traveled or the number of entries into enclosed arms between groups (fig. S7, A and B). In both of these tests, heterozygous $BDNF^{+/Met}$ mice did not display increases in anxiety-related behaviors (fig. S8). $BDNF^{+/-}$ mice also displayed increased anxiety-related behaviors in these two tests, similar in effect size to $BDNF^{Met/Met}$ mice (Fig. 3).

A common treatment for anxiety in humans are serotonin reuptake inhibitors (SSRIs), for which one postulated mechanism of action involves increasing BDNF levels (*17*). In rodents, this class of drugs increases hippocampal BDNF levels in a time frame corresponding to the onset of SSRI action (*18*) and is effective in decreasing anxiety-related behaviors in rodent models of mood disorders (*19*). $BDNF^{Met/Met}$ mice were treated orally with fluoxetine (18 mg/kg of body weight per day) or vehicle for 21 days before assessment in two tests: open field and novelty-induced hypophagia. This dosing regimen was based on prior studies showing that this dose led to a therapeutic serum levels (*19*). In the open-field test, fluoxetine led to a significant increase in time spent in the center for WT mice (Fig. 4A), as well as to an increase in entries into the center (fig. S9), which indicated its effectiveness in decreasing anxiety-related behaviors. However, there was a blunted response to fluoxetine in $BDNF^{Met/Met}$ mice, with respect to time spent in the center (Fig. 4A), as well as entries into the center (fig. S9). Furthermore, the reduction in exploration could not be explained by changes in locomotor activity (fig. S7A).

We used a specific behavioral paradigm for anxiety-related behavior, novelty-induced hypophagia, which has been suggested to be a more sensitive test of SSRI response (*20*, *21*). In this conflict test, mice are trained to approach a reward (sweetened milk) in their home cage and then placed in a novel brightly lit cage. The latency to approach and drink the sweetened milk is a measure of the anxiety-related behavior associated with this task (*20*). $BDNF^{Met/Met}$ mice treated with vehicle had a significantly greater latency to drink in the novel cage as compared with WT controls (Fig. 4B). Treatment with long-term fluoxetine did not significantly decrease the latency to drink in $BDNF^{Met/Met}$ mice, as in WT littermate mice treated in parallel with long-term fluoxetine (Fig. 4B). In both of these assays, $BDNF^{+/-}$ mice displayed similar diminished response to fluoxetine as compared with their WT controls (Fig. 4, A and B).

These results provide an example of a human SNP that has been modeled in transgenic mice that reproduce the same phenotypic hallmarks observed in Caucasian populations. Our subsequent analyses of these mice elucidated a phenotype that had not been established in human carriers: increased anxiety. When placed in

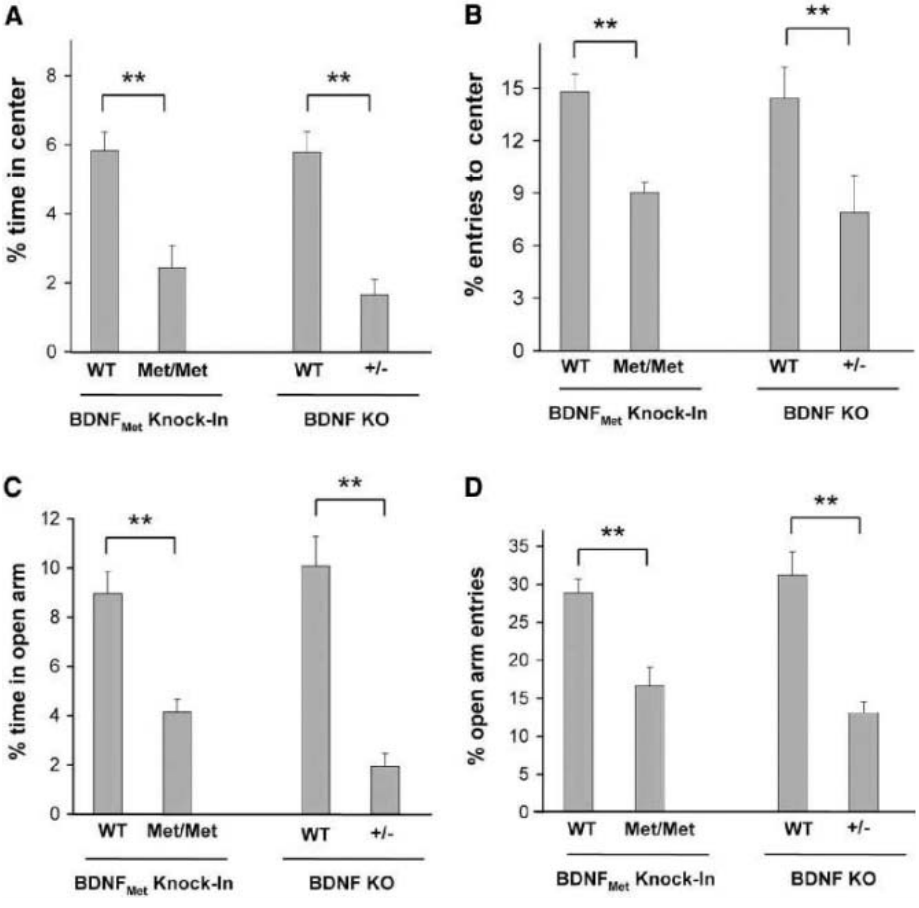


Fig. 3. Anxiety-related behavior in $BDNF^{Met/Met}$ mice in the open field (**A** and **B**) and elevated plus maze (**C** and **D**). Percentage of time spent in the center (A) and entries into the center (B) in the open field are shown, as well as percentage time spent in the open arm (C) and percentage of open arm entries (D) in the plus maze. All results are presented as means ± SEM determined from analysis of eight mice per genotype (**$P < 0.01$).

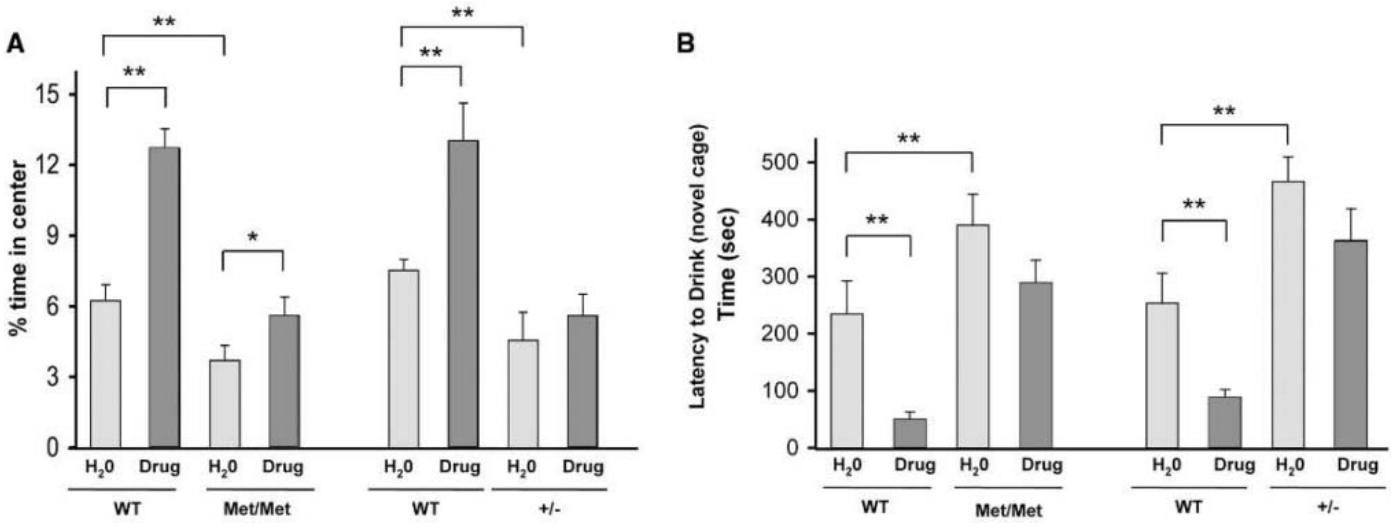


Fig. 4. Decreased response to long-term fluoxetine in BDNF$^{Met/Met}$ mice in the (**A**) open-field and (**B**) novelty-induced hypophagia tests. In the open-field test, percentage of time spent in the center in the absence (H_2O) or presence of fluoxetine (drug) treatment was measured. In the novelty-induced hypophagia test, latency to begin drinking in a novel cage in the absence (H_2O) or presence of fluoxetine (drug) treatment is shown in seconds. All results are presented as means ± SEM determined from analysis of eight mice per genotype (*$P < 0.05$, **$P < 0.01$).

conflict settings, BDNF$^{Met/Met}$ mice display increased anxiety-related behaviors in three separate tests and thus provide a genetic link between BDNF and anxiety. Genetic association studies have found that the Met allele has been associated with increased trait anxiety (*22*), but other studies have not replicated these findings (*23*–*25*). Two main differences in our study design led to discerning this anxiety-related phenotype. First, mice were subjected to conflict tests to elicit the increased anxiety-related behavior, whereas human studies relied on questionnaires. Second, the anxiety-related phenotype was only present in mice homozygous for the Met allele, which suggested that association studies that focused primarily on humans heterozygous for the Met allele may not detect an association. In this context, another human genetic polymorphism in the serotonin transporter (5HTLPR) was associated with depression only in homozygote subjects with past trauma histories (*26*), which suggested that environmental influences, as well as gene dosage, were required for this SNP to influence psychiatric pathology.

The form of anxiety elicited in these BDNF$^{Met/Met}$ mice was not responsive to a common SSRI. It has previously been shown in conditional BDNF KO mice that there is altered expression or function of serotonin receptors (*27*–*29*). These results suggest that humans with this allele may not have optimal responses to this class of antidepressants. Currently, there are no reliable genetic or nongenetic biomarkers to predict who will respond to an SSRI. The transgenic BDNF$^{Met/Met}$ mouse may serve as a valuable model to identify novel pharmacologic approaches to treating anxiety disorders.

Finally, the BDNF$^{Met/Met}$ mouse represents a unique model that directly links activity-dependent release of BDNF to a defined set of in vivo consequences. In these mice, $BDNF_{Met}$ expression is equivalent to that of BDNF expression in WT controls (Fig. 1C), but there is an ~30% deficit in activity-dependent release of $BDNF_{Met}$ from neurons (Fig. 1D), that contributes to a specific set of anatomical and behavioral deficits. Because BDNF$^{Met/Met}$ mice are similar to BDNF$^{+/-}$ mice in that they have BDNF levels that are lower by 50%, it is possible that there is a threshold level of lowered BDNF that is crossed by both mutant mice. It also remains possible that there are additional defects in $BDNF_{Met}$ processing that may contribute to the observed deficits, although in vitro studies in neurons suggest no defect in $BDNF_{Met}$ processing (*6*, *8*). In all, these findings indicate a new direction in therapeutic strategies to rescue anxiety symptoms in humans with this polymorphic allele. Drug discovery strategies to increase BDNF release from synapses or to prolong the half life of secreted BDNF may improve therapeutic responses for humans with this common BDNF polymorphism.

References and Notes

1. E. Shimizu, K. Hashimoto, M. Iyo, *Am. J. Med. Genet. B. Neuropsychiatr. Genet.* **126B**, 122 (2004).
2. J. A. Bueller *et al.*, *Biol. Psychiatry* **59**, 812 (2006).
3. L. Pezawas *et al.*, *J. Neurosci.* **24**, 10099 (2004).
4. P. R. Szeszko *et al.*, *Mol. Psychiatry* **10**, 631 (2005).
5. A. R. Hariri *et al.*, *J. Neurosci.* **23**, 6690 (2003).
6. M. F. Egan *et al.*, *Cell* **112**, 257 (2003).
7. Z. Y. Chen *et al.*, *J. Neurosci.* **25**, 6156 (2005).
8. Z. Y. Chen *et al.*, *J. Neurosci.* **24**, 4401 (2004).
9. B. Lu, *Neuron* **39**, 735 (2003).
10. J. A. Bueller *et al.*, *Biol. Psychiatry* **59**, 812 (2006).
11. F. Caserta *et al.*, *J. Neurosci. Methods* **56**, 133 (1995).
12. I. Y. Liu, W. E. Lyons, L. A. Mamounas, R. F. Thompson, *J. Neurosci.* **24**, 7958 (2004).
13. M. Rios *et al.*, *Mol. Endocrinol.* **15**, 1748 (2001).
14. L. M. Monteggia *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **101**, 10827 (2004).
15. W. E. Lyons *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **96**, 15239 (1999).
16. B. S. McEwen, *Ann. N.Y. Acad. Sci.* **933**, 265 (2001).
17. R. S. Duman, G. R. Heninger, E. J. Nestler, *Arch. Gen. Psychiatry* **54**, 597 (1997).
18. M. Nibuya, S. Morinobu, R. S. Duman, *J. Neurosci.* **15**, 7539 (1995).
19. S. C. Dulawa, K. A. Holick, B. Gundersen, R. Hen, *Neuropsychopharmacology* **29**, 1321 (2004).
20. S. C. Dulawa, R. Hen, *Neurosci. Biobehav. Rev.* **29**, 771 (2005).
21. L. Santarelli *et al.*, *Science* **301**, 805 (2003).
22. X. Jiang *et al.*, *Neuropsychopharmacology* **30**, 1353 (2005).
23. U. E. Lang *et al.*, *Psychopharmacology (Berlin)* **180**, 95 (2005).
24. S. Sen *et al.*, *Neuropsychopharmacology* **28**, 397 (2003).
25. P. G. Surtees *et al.*, *J. Psychiatr. Res.*, published online 10.1016/j.jpsychires.2006.05.015 (25 July 2006).
26. A. Caspi *et al.*, *Science* **301**, 386 (2003).
27. J. P. Chan, T. J. Unger, J. Byrnes, M. Rios, *Neuroscience*, published online 10.1016/j.neuroscience.2006.06.002 (13 July 2006).
28. M. Rios *et al.*, *J. Neurobiol.* **66**, 408 (2006).
29. J. G. Hensler, E. E. Ladenheim, W. E. Lyons, *J. Neurochem.* **85**, 1139 (2003).

30. Supported by the National Alliance for Research on Schizophrenia and Depression (F.S.L., Z.-Y.C., D.G.H.); DeWitt-Wallace Fund of the New York Community Trust (F.S.L., D.G.H.); Nancy Pritzker Depression Network (F.S.L., D.G.H.); Sackler Institute (F.S.L.); Foundation for the author of National Excellent Doctoral Dissertation of People's Republic of China (No. 200229) (Z.-Y.C.); Shanghai Rising-Star Program (05QMH1401) (Z.-Y.C.); Taishan Scholar Program (Z.-Y.C.); National Key Basic Research Program 2006CB503800 (Z.-Y.C.); and NIH (NS052819 to F.S.L., MH068850 to F.S.L., NS30687 to B.L.H., and MH060478 to K.G.B.). We thank M. Chao, L. Tessarollo, F. Maxfield, C. Dreyfus, C. Glatt, P. Perez, and K. Teng for helpful discussions.

Supporting Online Material

www.sciencemag.org/cgi/content/full/314/5796/140/DC1
Materials and Methods
Figs. S1 to S9
References and Notes

8 May 2006; accepted 16 August 2006
10.1126/science.1129663

Activity- and mTOR-Dependent Suppression of Kv1.1 Channel mRNA Translation in Dendrites

Kimberly F. Raab-Graham,* Patrick C. G. Haddick,* Yuh Nung Jan, Lily Yeh Jan†

Mammalian target of rapamycin (mTOR) is implicated in synaptic plasticity and local translation in dendrites. We found that the mTOR inhibitor, rapamycin, increased the Kv1.1 voltage-gated potassium channel protein in hippocampal neurons and promoted Kv1.1 surface expression on dendrites without altering its axonal expression. Moreover, endogenous Kv1.1 mRNA was detected in dendrites. Using Kv1.1 fused to the photoconvertible fluorescence protein Kaede as a reporter for local synthesis, we observed Kv1.1 synthesis in dendrites upon inhibition of mTOR or the *N*-methyl-D-aspartate (NMDA) glutamate receptor. Thus, synaptic excitation may cause local suppression of dendritic Kv1 channels by reducing their local synthesis.

Pathways involving the mTOR kinase and its upstream phosphoinositide 3-kinase (phosphatidylinositol 3-kinase, or PI3K) are important for neuronal signaling, including long-term potentiation (LTP) and long-term depression (LTD) (*1*–*4*). Negative regulators of this pathway, tumor suppressors linked to tuberous sclerosis complex (TSC) and Peutz-Jeghers syndrome (*5*), regulate local synthesis in synaptodendritic compartments (*4*, *6*–*8*). Whether the mTOR pathway regulates ion channel synthesis and localization is an interesting open question. Although Kv1 channels are primarily localized to axons (*9*), they also contribute to the rapidly inactivating (A-type) and slowly inactivating (D-type) Kv channels in somatodendritic regions of neurons in the brain [(*10*) and references therein], thereby controlling local excitability of dendritic branches (*11*, *12*) and restricting calcium spike generation to dendrites (*13*). Whereas the T1 tetramerization domain mediates Kv1 axonal targeting (*14*), it is unknown whether specific mechanisms are used for dendritic localization of Kv1 channels.

We found that rapamycin increased Kv1.1 (by 50 ± 14%, $P = 0.03$, $n = 3$) but not Kv1.4 in the CA1 dendritic field of rat hippocampal slices (Fig. 1A and fig. S1A). Rapamycin also increased Kv1.1 in cortical and hippocampal neuronal cultures (Fig. 1B and fig. S1B). Consistent with mTOR activation by PI3K and its effector Akt/protein kinase B thereby causing phosphorylation of cap-dependent translation initiation factors and the p70 S6 kinase (*6*, *15*), rapamycin reduced phosphorylation of p70 S6 kinase but not Akt (Fig. 1B and fig. S1B). Whereas mTOR activity is generally associated with increased protein synthesis, it can also down-regulate certain proteins (*2*, *16*–*18*), similar to the down-regulation of Kv1.1 here.

In cultured hippocampal neurons expressing the microtubule-associated protein MAP2 in dendrites (Fig. 1C) and neural filament M in axons (fig. S1C), rapamycin increased Kv1.1 in punctate structures in dendrites but not axons (Fig. 1C). These Kv1.1-containing puncta partially (60 ± 5%) colocalized with mTOR-containing granules (fig. S2B). Moreover, antibody that recognizes an extracellular epitope of Kv1.1 (*19*) revealed high levels of Kv1.1 on the surface of proximal apical dendrites in neurons treated with rapamycin but not in control neurons (Fig. 1D).

To determine whether Kv1.1 could be synthesized in dendrites, we first localized endogenous Kv1.1 mRNA to dendrites by in situ hybridization (Fig. 2A and fig. S2A). Our microarray analysis revealed that, for Kv1.1 mRNA, the level in synaptosomes normalized to that in hippocampi was greater than those for the dendritically targeted MAP2 and ARC (activity-regulated cytoskeletal associated protein) mRNA and substantially higher than those for transcripts for the synaptic protein NSF (*N*-ethylmaleimide–sensitive factor) and nuclear proteins such as histones (table S1). Similarly, the normalized ratio for Kv1.1 mRNA determined by real-time polymerase chain reaction (PCR) was comparable to that for MAP2 mRNA (*20*) (Fig. 2B). Moreover, in hippocampal neurons infected with a Sindbis viral construct programmed to express fluorescent Kv1.1, the exogenous Kv1.1 mRNA including the 3′ untranslated region (3′UTR) was present in granules in dendrites up to 110 μm from the soma (Fig. 2C).

Next, we devised a method to distinguish Kv1.1 synthesized in dendrites from Kv1.1 synthesized in soma and then transported to dendrites. We fused Kaede, a photoconvertible fluorescent protein (*21*), to the N terminus of Kv1.1 (Kaede-Kv1.1), followed with the 3′UTR of Kv1.1 (fig. S3). Ultraviolet (UV) light induces cleavage of Kaede, converting its fluorescence from green into red. Newly synthesized Kaede-Kv1.1 could then be identified by its green chromophore. To look for newly synthesized Kaede-Kv1.1 in Sindbis virus–infected neurons treated with rapamycin or PI3K inhibitors, we imaged live neurons before and after UV-induced photoconversion of Kaede-Kv1.1 into red fluorescent protein and observed newly synthesized Kaede-Kv1.1 appearing as green fluorescence over 1 hour, before a second UV exposure photoconverted newly synthesized Kaede-Kv1.1—but not background fluorescence—into red fluorescence. We found that treating neurons with rapamycin, or with the PI3K inhibitors wortmannin or LY294002, caused a marked increase in newly synthesized Kaede-Kv1.1 in dendrites (Fig. 3A). This was due to local synthesis because there was little movement of Kaede-Kv1.1: After photoconversion of the Kaede fluorescence within a dendritic branch, there was no detectable green Kaede-Kv1.1 in that region over 50 min, whereas Kaede fused to only the soluble N-terminal domain of Kv1.1 would have moved to the photoconverted dendritic region within 5 min (fig. S3). In rare occasions we observed Kaede-Kv1.1 and EGFP-Kv1.1 (Kv1.1 fused to enhanced green fluorescent protein) moving with the slow transport rate of less than 15 μm/hour. In general, the fluorescently tagged Kv1.1 in dendrites was concentrated in stationary "hotspots," as reported for locally translated proteins (*8*, *22*).

After photoconversion, the newly synthesized Kaede-Kv1.1 grew over 1 hour at preexisting hotspots without detectable movements (Fig. 3A, arrows). The newly synthesized Kaede-Kv1.1 that appeared in dendritic puncta more than 50 μm from the soma was increased by a factor of 3 in the presence of rapamycin or the PI3K inhibitors LY294002 or wortmannin (Fig. 3B). The protein synthesis inhibitors anisomycin and cycloheximide reduced this rapamycin effect (Fig. 4, B and C), supporting the notion of Kv1.1 local synthesis in the dendrites.

To explore how Kv1.1 local translation might be regulated by neuronal activity, we examined neuronal cultures with and without treatments with antagonists of the *N*-methyl-D-aspartate (NMDA) glutamate receptor and metabotropic glutamate receptor. The NMDA receptor antagonist AP5 significantly increased endogenous Kv1.1 but decreased mTOR phosphorylation, raising the ratio of Kv1.1 protein to phosphorylated mTOR (P-mTOR) protein by a factor of 11 (Fig. 4A), a process that required protein synthesis (Fig. 4, B and C). It thus appears that NMDA receptors activated as a result of synaptic activity could locally regulate Kv1.1 synthesis near the active synapse.

We note that Kv1.1 mRNA does not contain internal ribosome entry sites (IRES) implicated in mTOR regulation, and the 5′UTR of Kv1.1 was

Howard Hughes Medical Institute, Departments of Physiology and Biochemistry, University of California, San Francisco, CA 94158, USA.

*These authors contributed equally to this work.
†To whom correspondence should be addressed. E-mail: lily.jan@ucsf.edu

not required for Kaede-Kv1.1 local synthesis regulation. The 3′UTR of Kv1.1 mRNA has two putative AU-rich elements (AREs) similar to those in the ligatin mRNA under NMDA receptor regulation (*23*). ARE binding proteins could either stabilize mRNA for translation or promote mRNA degradation, and they compete with one another for mRNA binding (*24*). It remains to be determined whether NMDA receptor activation shifts Kv1.1 mRNA from a form readily accessible to translation to a different complex for silencing or degradation.

How might NMDA receptors activate PI3K and mTOR to suppress Kv1.1? LTP is associated with regional increase of PI3K activity (*25*), probably because of NMDA receptor–mediated Ca^{2+} influx that activates PI3K associated with AMPA glutamate receptors nearby (*26*), and causes Akt and p70 S6 kinase phosphorylation essential for LTP expression but not induction or maintenance (*25*). NMDA receptor activation of PI3K at the active synapses could suppress Kv1.1 synthesis locally in the dendrites and may enhance excitatory postsynaptic potential (EPSP) spike potentiation (*12*).

NMDA receptor–mediated suppression of Kv1.1 local synthesis is a positive feedback mechanism that could specifically potentiate active synapses by enhancing voltage-gated sodium and/or calcium channel activation during EPSP, thus facilitating EPSP summation and action potential generation (*11*, *27*) (fig. S5). Hippocampal neuronal dendrites exhibit uneven distribution of Kv channels (*11*, *28*), one-fifth of which is sensitive to the Kv1-specific α-dendrotoxin (*11*), to prevent action potential initiation in dendrites and dampen backpropagation (*11*, *13*). With the intriguing findings of clustered D-type Kv channels on CA1 dendrites (*28*) and localized increase of dendritic excitability and calcium influx within the dendritic branch with potentiated excitatory postsynaptic

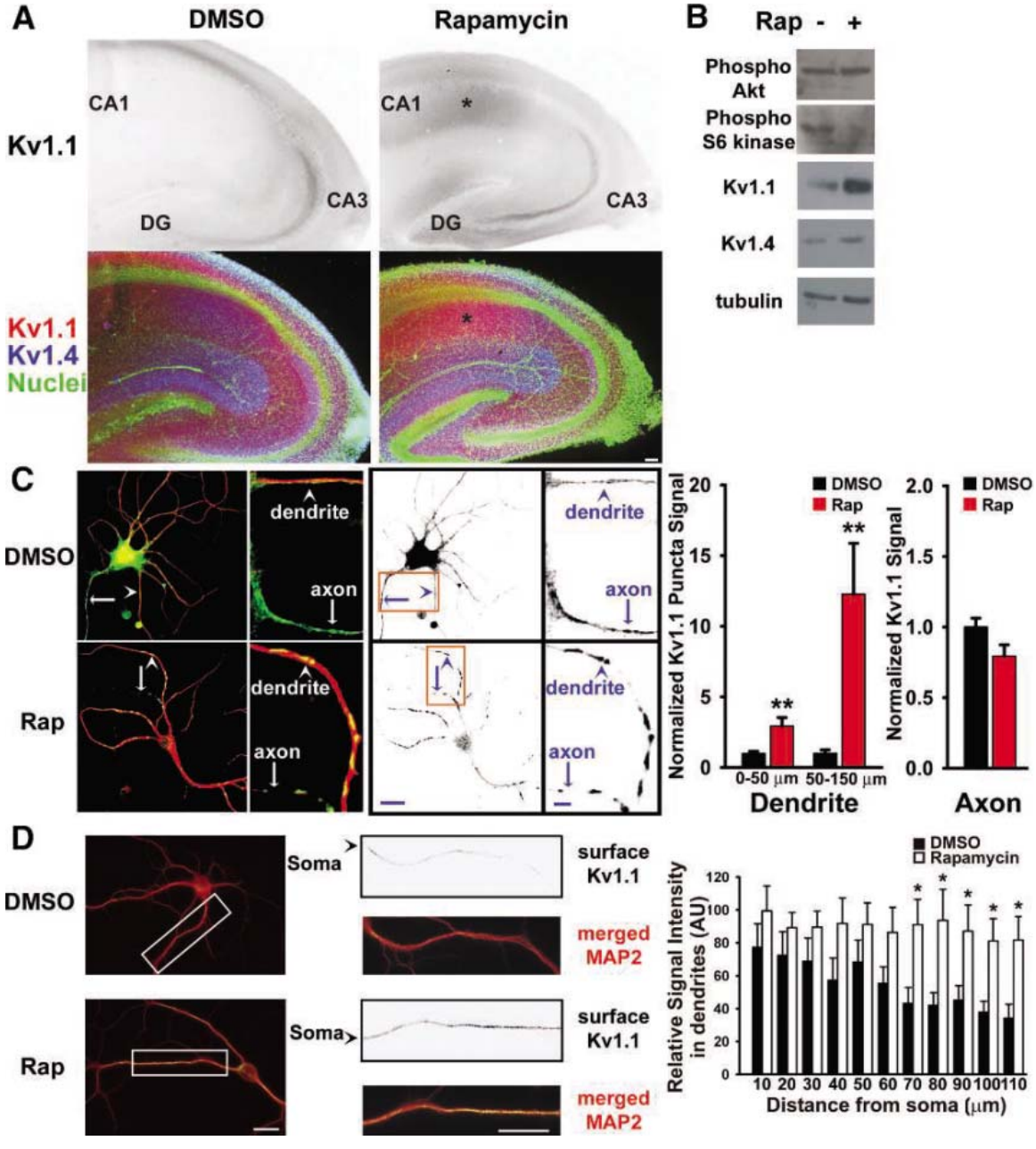


Fig. 1. Rapamycin inhibition of mTOR increased Kv1.1 protein in central neuronal dendrites. (**A**) Kv1.1 (black, upper panel; red, lower panel) and Kv1.4 (blue, lower panel) distribution in hippocampal slices stained with the DNA dye SYTOX (green) after treatment with dimethyl sulfoxide (DMSO; control) or rapamycin (200 nM) for 75 min in oxygenated artificial cerebrospinal fluid (ACSF) at 37°C. Scale bar, 100 μm. DG, dentate gyrus. (**B**) Immunoblot of phospho-Akt, phospho-S6 kinase, Kv1.1, Kv1.4, and tubulin of cultured cortical neurons [days in vitro (DIV) 30] with or without exposure to rapamycin. (**C**) Left: Cultured hippocampal neurons (DIV 23) treated with DMSO (control) or rapamycin (Rap; 200 nM) double-labeled for Kv1.1 (green) and MAP2 (red). Scale bar, 20 μm. Right: Rapamycin increased Kv1.1 level in dendritic puncta; $^{**}P < 0.005$. (**D**) Left: Surface Kv1.1 of unpermeabilized hippocampal neurons (DIV 21) in DMSO (control) or rapamycin (200 nM) was labeled with antibody to an extracellular epitope of Kv1.1 (green), followed by permeabilization and MAP2 staining (red). Scale bar, 20 μm. The Kv1.1 puncta in permeabilized neurons (C) were "hotspots" that partially colocalized with mTOR granules (fig. S2B). Right: Rapamycin increased Kv1.1 surface expression in distal dendrites; $^{*}P < 0.05$. See SOM for quantifications for (C) and (D).

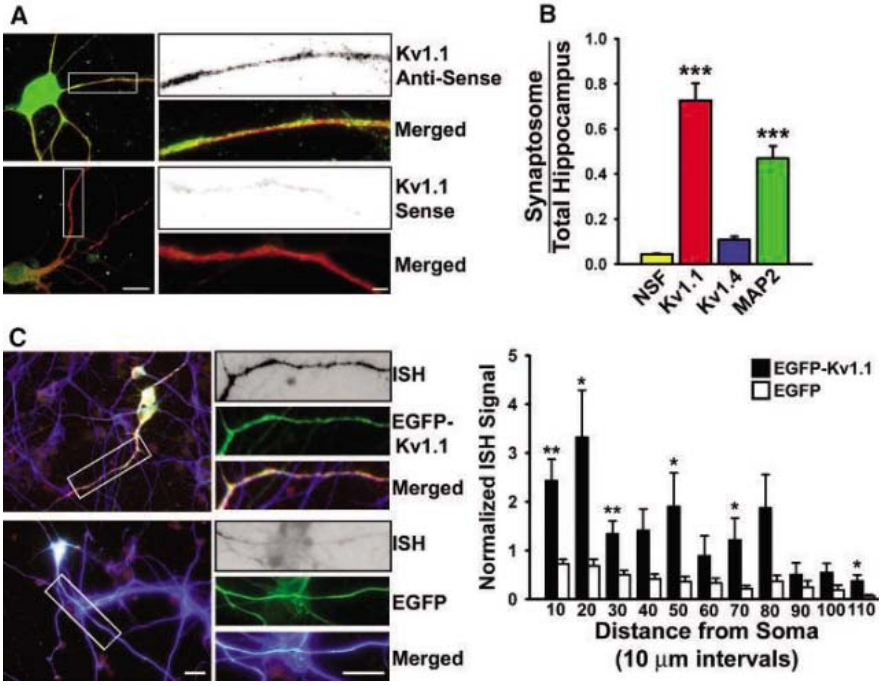
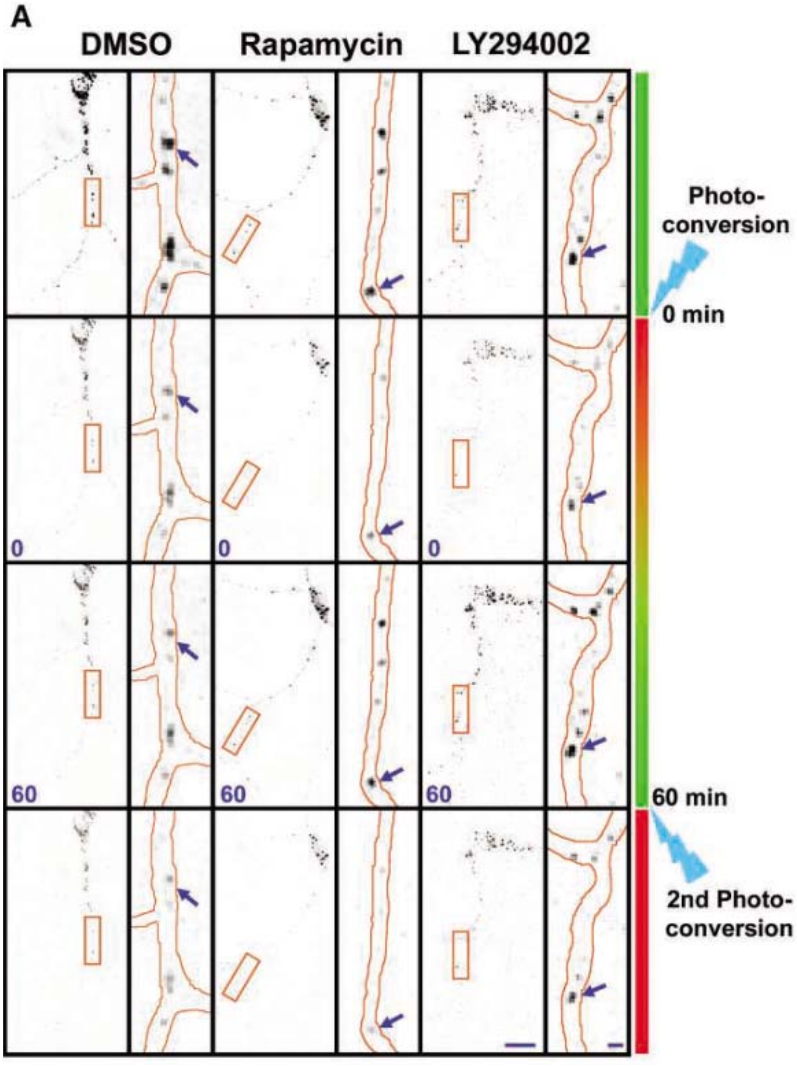


Fig. 2. Kv1.1 mRNA in hippocampal neuronal dendrites. (**A**) Kv1.1 mRNA in MAP2-positive (red) dendrites of hippocampal neurons (DIV 28) revealed by in situ hybridization using antisense (top) and sense control (bottom) probe against 3′UTR of Kv1.1 (green); scale bar, 20 μm. Boxed dendrites in left panels are shown at high magnification on the right; scale bar, 5 μm. (**B**) Real-time PCR revealed that the ratio of synaptosomal mRNA and total hippocampal mRNA (synaptosome/total hippocampus) for Kv1.1 and MAP2, but not Kv1.4, was greater than that for NSF. ***$P < 0.001$; quantifications given in SOM. (**C**) Left: Dendritic localization of EGFP-Kv1.1 RNA (top), but not EGFP RNA, both with polyadenylation sequence, revealed by in situ hybridization (ISH) using the same antisense probe for EGFP (DIV 21). The boxed dendrites are shown at high magnification on the right. RNA (ISH), black in top panel, red in merged panel; EGFP, green; MAP2, blue. Scale bar, 20 μm. Right: Normalized ISH signal of EGFP-Kv1.1 mRNA (solid bars) and EGFP mRNA (open bars). Mean signal intensities were averaged every 10 μm along the dendrite (end point indicated on the *x* axis) and divided by hybridization signals within the first 10 μm from the soma to normalize for expression. Error bars represent SEM; *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ (Student's *t* test for comparison with EGFP control, $n = 13$ dendrites).



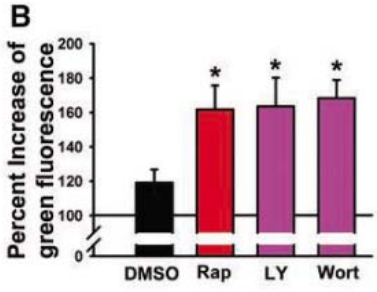


Fig. 3. Inhibition of PI3K or mTOR increased local translation of Kaede-Kv1.1. (**A**) Live imaging of neurons in ACSF containing DMSO (control), rapamycin (200 nM), LY294002 (50 μM), or wortmannin (50 nM) before, immediately after (0 min), and 60 min after the first UV exposure to photoconvert Kaede-Kv1.1 into red fluorescent protein, for appearance of newly synthesized green Kaede-Kv1.1, which was then turned into red by the second photoconversion. Left panels: Representative grayscale images of green fluorescence in neurons. Scale bar, 20 μm. Right panels: Dendrite in orange boxed region with arrow pointing to a single Kaede-Kv1.1 puncta. Scale bar, 2 μm. (**B**) Normalized newly synthesized Kaede-Kv1.1 (green fluorescence pixel intensity) in individual puncta more than 50 μm from the soma, over the course of 1 hour, was increased by rapamycin, LY, or wortmannin. *$P < 0.05$; quantifications given in SOM.

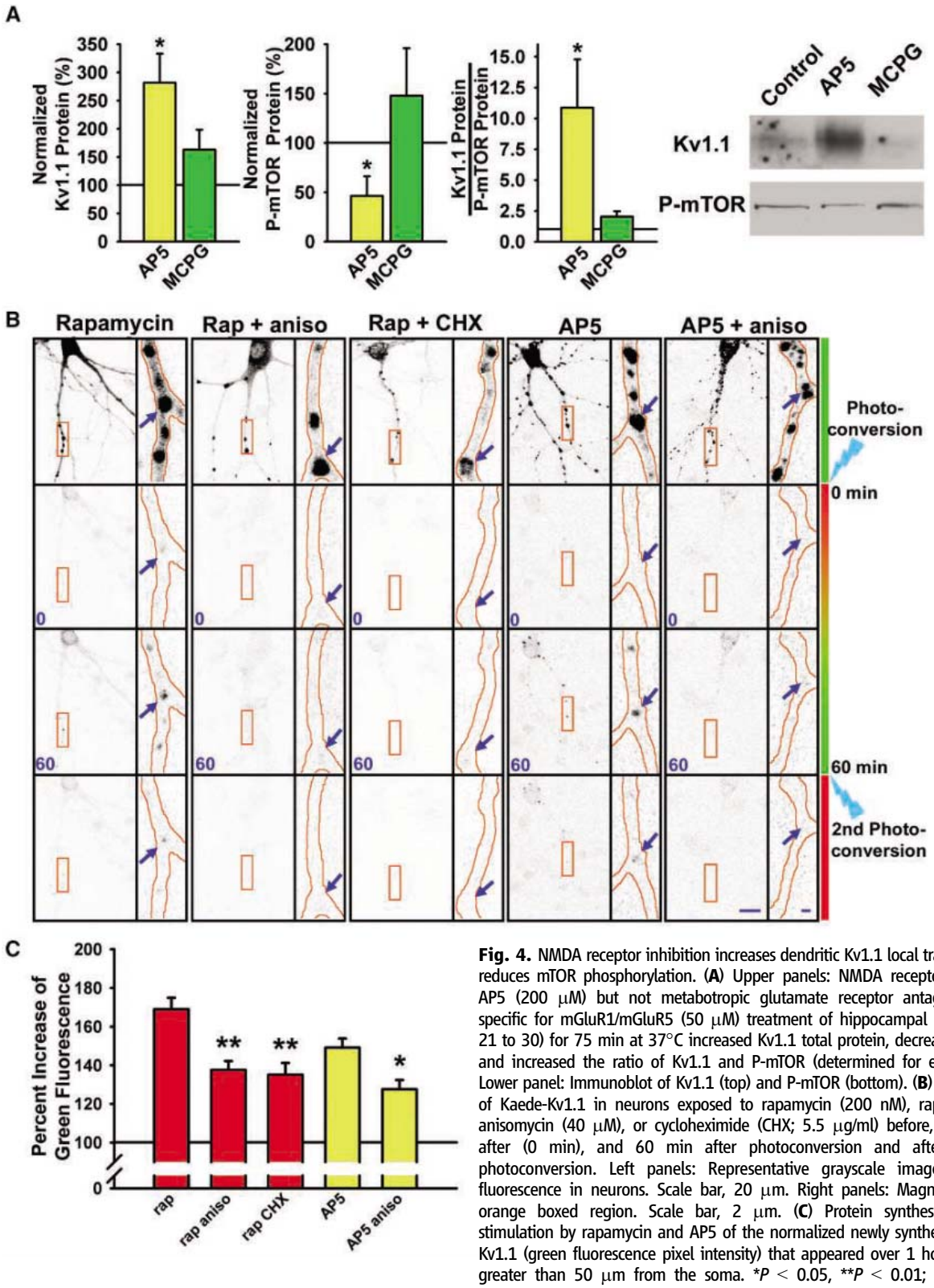


Fig. 4. NMDA receptor inhibition increases dendritic Kv1.1 local translation and reduces mTOR phosphorylation. (**A**) Upper panels: NMDA receptor antagonist AP5 (200 μM) but not metabotropic glutamate receptor antagonist MCPG specific for mGluR1/mGluR5 (50 μM) treatment of hippocampal neurons (DIV 21 to 30) for 75 min at 37°C increased Kv1.1 total protein, decreased P-mTOR, and increased the ratio of Kv1.1 and P-mTOR (determined for each sample). Lower panel: Immunoblot of Kv1.1 (top) and P-mTOR (bottom). (**B**) Live imaging of Kaede-Kv1.1 in neurons exposed to rapamycin (200 nM), rapamycin plus anisomycin (40 μM), or cycloheximide (CHX; 5.5 μg/ml) before, immediately after (0 min), and 60 min after photoconversion and after a second photoconversion. Left panels: Representative grayscale images of green fluorescence in neurons. Scale bar, 20 μm. Right panels: Magnified view of orange boxed region. Scale bar, 2 μm. (**C**) Protein synthesis–dependent stimulation by rapamycin and AP5 of the normalized newly synthesized Kaede-Kv1.1 (green fluorescence pixel intensity) that appeared over 1 hour in puncta greater than 50 μm from the soma. $*P < 0.05$, $**P < 0.01$; see SOM for quantifications for (A) and (C).

current (*12*), it is an interesting open question whether synaptic activities dynamically regulate the size and location of microdomains of Kv1 channels, thereby influencing integration and plasticity of synaptic inputs near recently active synapses to adjust the quality and capacity of information storage (*27*). It will also be important to elucidate homeostasis mechanisms of Kv1.1 local synthesis regulation for the resetting of local excitability.

References and Notes

1. L. Hou, E. Klann, *J. Neurosci.* **24**, 6352 (2004).
2. E. Klann, T. E. Dever, *Nat. Rev. Neurosci.* **5**, 931 (2004).
3. S. J. Tang *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **99**, 467 (2002).

4. M. Cammalleri *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **100**, 14368 (2003).
5. K. Inoki, M. N. Corradetti, K. L. Guan, *Nat. Genet.* **37**, 19 (2005).
6. N. Takei *et al.*, *J. Neurosci.* **24**, 9760 (2004).
7. G. M. Schratt, E. A. Nigh, W. G. Chen, L. Hu, M. E. Greenberg, *J. Neurosci.* **24**, 7366 (2004).
8. G. Aakalu, W. B. Smith, N. Nguyen, C. Jiang, E. M. Schuman, *Neuron* **30**, 489 (2001).
9. J. S. Trimmer, K. J. Rhodes, *Annu. Rev. Physiol.* **66**, 477 (2004).
10. D. Guan *et al.*, *J. Physiol.* **571**, 371 (2006).
11. D. A. Hoffman, J. C. Magee, C. M. Colbert, D. Johnston, *Nature* **387**, 869 (1997).
12. A. Frick, J. Magee, D. Johnston, *Nat. Neurosci.* **7**, 126 (2004).
13. N. L. Golding, H. Y. Jung, T. Mickus, N. Spruston, *J. Neurosci.* **19**, 8789 (1999).
14. C. Gu, Y. N. Jan, L. Y. Jan, *Science* **301**, 646 (2003).
15. E. J. Brown, S. L. Schreiber, *Cell* **86**, 517 (1996).
16. L. Pirola *et al.*, *J. Biol. Chem.* **278**, 15641 (2003).
17. F. Gebauer, M. W. Hentze, *Nat. Rev. Mol. Cell Biol.* **5**, 827 (2004).
18. T. Peng, T. R. Golub, D. M. Sabatini, *Mol. Cell. Biol.* **22**, 5575 (2002).
19. A. M. Tiffany *et al.*, *J. Cell Biol.* **148**, 147 (2000).
20. A. Blichenberg *et al.*, *J. Neurosci.* **19**, 8818 (1999).
21. H. Mizuno *et al.*, *Mol. Cell* **12**, 1051 (2003).
22. O. Steward, E. M. Schuman, *Neuron* **40**, 347 (2003).
23. E. R. Jakoi, D. M. Panchision, C. M. Gerwin, R. J. DeLorenzo, *Brain Res.* **693**, 124 (1995).
24. C. Barreau, L. Paillard, H. B. Osborne, *Nucleic Acids Res.* **33**, 7138 (2005).
25. P. P. Sanna *et al.*, *J. Neurosci.* **22**, 3359 (2002).
26. H. Y. Man *et al.*, *Neuron* **38**, 611 (2003).
27. A. Frick, D. Johnston, *J. Neurobiol.* **64**, 100 (2005).
28. X. Chen, D. Johnston, *Pflugers Arch.*, 10.1007/s00424-006-0097-y (2006).
29. We thank J. Trimmer for Kv1.1 antibodies, A. Miyawaki for the Kaede construct, and S. Yurkovskaya and M. Jain for technical assistance. Supported by National Institute of Mental Health grant MH65334 (L.Y.J.) and by Individual NIH Research Service Award in Mental Health MH13010 (K.F.R.-G.). Y.N.J. and L.Y.J. are HHMI investigators.

Supporting Online Material

www.sciencemag.org/cgi/content/full/314/5796/144/DC1
Materials and Methods
Figs. S1 to S5
Table S1
References

23 June 2006; accepted 11 August 2006
10.1126/science.1131693

TIRF System

The Leica AM TIRF (total internal reflection fluorescent) System combines superior fluorescence imaging performance, high sensitivity, and an optimum signal-to-noise ratio to ensure accurate results of all examinations of close-to-membrane structures. TIRF is a particularly useful imaging technique for visualizing vesicle transport, interaction between molecules, and membrane dynamics. The evanescent illumination field from the TIRF objective penetrates specimens only a few nanometers, resulting in high signal-to-noise ratios at the plane of focus. The Leica AM system offers absolute assurance that TIRFF is achieved with automatic laser alignment, a large field of view, complete control of the evanescent wave penetration depth and propagation direction, and an objective that features color correction and variable temperature correction from 23° to 37° C.
Leica Microsystems For information 800-248-0123 www.leica-microsystems.us

Circular ssDNA

CircLigase ssDNA Ligase is a thermostable, ATP-dependent ligase that efficiently catalyzes circularization of single-stranded DNA (ssDNA) with 5′-phosphate and 3′-hydroxy termini. CircLigase does not require oligonucleotide "splints" or "bridges" and produces no detectable linear or circular concatamers, making the enzyme suitable for preparing circular ssDNA templates for rolling circle replication or rolling circle transcription.
Epicentre Biotechnologies For information 800-284-8474 www.epibio.com/CircLigase.asp

Higher Modified Protein Yields

Many eukaryotic proteins require posttranslational modifications (PTMs) to be able to fold or function correctly. For efficient expression of a broad range of eukaryotic proteins with PTMs, the EasyXpress Insect Kit II makes use of an optimized lysate production procedure to provide higher yields. It features highly productive *Spodoptera frugiperda* lysates, which contain everything required for protein expression and PTMs (e.g., enzymes, protein assemblies, membrane fractions, and ribosomes). In trials, the new kit enabled glycosylation, phosphorylation, or signal-peptide cleavage of numerous proteins, from 14 to 115 kDa.
Qiagen For information 800-426-8157. www.qiagen.com

RNA from Tissue

The Agencourt RNAdvance Tissue system for isolation of total RNA from mammalian samples is based on the patented Agencourt SPRI (solid-phase reversible immobilization) paramagnetic bead nucleic acid purification technology. The new system produces clean RNA with high recovery, in single-tube or 96-well formats, while eliminating organic solvents, vacuum filtration, and centrifugation. The simple procedure enables higher recovery of total RNA than traditional column-based approaches, which allows researchers to maximize the use of precious tissue samples without compromising RNA integrity. The process can be automated on the Beckman Coulter Biomek NX liquid handling workstation. For microarray applications, the system can be used with the Agencourt RNAClean technology.
Beckman Coulter For information 978-867-2922 www.agencourt.com

High-Throughput Plasmid Screening

Toothpick-PCR allows for the rapid release of plasmids from transformed bacteria for screening by polymerase chain reaction (PCR). There is no requirement for growing bacteria, performing "minipreps," or purifying the plasmid DNA. The user adds a colony directly to the supplied Toothpick solution, heats it, and then transfers 1 μl directly to a PCR reaction to screen for a plasmid. Following PCR, Glow Loading Dye is added and an agarose gel run. There is no need to stain with ethidium bromide; the gel can be placed in an ultraviolet light box and viewed.
G-Biosciences/Genotech For information 800-628-7730 www.GBiosciences.com

Mouse Models for Research

A new embryonic stem cell line, the Hybrid 129xC57 line, exhibits a significantly higher rate of homologous recombination in the production of mouse knockout and knockin models. The Hybrid 129xC57 has shown an average 200% increase in the frequency of positive clones per targeting event than other cell lines used at inGenious Targeting Laboratory (iTL), a contract research facility that produces genetically modified mice for pharmaceutical, biotechnology, academic, and government institutions. These characteristics have resulted in reducing the time involved for electroporation and screening tasks, enabling delivery of mouse models with guaranteed germline transmission to be achieved in as little as seven months. The Hybrid 129xC57 line is the third embryonic stem cell line developed by iTL, and is designed to incorporate the strengths of its 129SvEv and C57BL/6 lines. The 129SvEv line exhibits a consistent rate of homologous recombination for efficient germline transmission while enabling the ability to analyze resulting chimeras by coat color. The C57BL/6 line is useful for its physiological relevance to certain genetically linked health conditions, its many parallels between mouse and human genes, the accuracy of published databases for C57BL/6 genes, and the ability to obtain a mouse on a pure background.
inGenious Targeting Laboratory For information 631-444-6828 www.genetargeting.com

Newly offered instrumentation, apparatus, and laboratory materials of interest to researchers in all disciplines in academic, industrial, and government organizations are featured in this space. Emphasis is given to purpose, chief characteristics, and availability of products and materials. Endorsement by *Science* or AAAS of any products or materials mentioned is not implied. Additional information may be obtained from the manufacturer or supplier by visiting www.science.labvelocity.com on the Web, where you can request that the information be sent to you by e-mail, fax, mail, or telephone.

EXIQON

microRNA profiling service

using miRCURY™ LNA Arrays

Obtain the miRNA profiles of your samples using:

- **miRCURY™ LNA Arrays**
- **Exiqon's time and expertise**

What could be easier?

Contact Exiqon to learn more

United States
Phone: +1 781 376 4150
Email: support@exiqon.com

Rest of the World
Phone: +45 45 650 929
Email: support@exiqon.com

www.exiqon.com

Life Science Technologies

Aging and Neuroscience

PROBING THE BRAIN

Researchers face huge challenges as they seek to discover the causes and mechanisms of age-related neurological ailments such as Alzheimer's and Parkinson's diseases, and to translate their findings into effective methods of diagnosis and treatment. But emerging tools and technologies have begun to unravel some of the conditions' complexities. **by Peter Gwynne and Gary Heebner**

Neurological ailments that afflict patients as they age, such as Alzheimer's disease and Parkinson's disease, present medical scientists with one of their greatest challenges. While research continually increases knowledge of aging and related diseases, many of the details of how the brain works (and doesn't work) remain a mystery.

However, researchers have begun to make significant advances in recent years. Those include the discovery that new nerve cells can be made in the brain and spinal cord, the identification of biomarkers that can help to detect Alzheimer's disease before clinical diagnosis and signs of memory loss appear, and the finding that a mutation in a recently discovered gene (LRRK2) is the most common genetic cause of Parkinson's disease.

Plainly, studies of neurological diseases related to the aging process have begun to gather momentum. "The field is really fermenting these days," says Marcelle Morrison-Bogorad, director of the neuroscience and neuropsychology of aging program at the **U.S. National Institute on Aging**. "We are trying to accelerate the process of moving from basic research on pathways to translational science and taking the next step of deciding what compounds are at a stage when we can put them into clinical trials." Scientists who attended July's International Conference on Alzheimer's Disease and Related Disorders in Madrid, Spain, expressed similar optimism. "A lot of people said that this is the beginning of an important time for Alzheimer's," recalls Ron Black, senior director of neuroscience medical research at **Wyeth**.

IN THIS ISSUE:

Tools Old and New

To fulfill that promise, researchers rely on a variety of tools and technologies – both traditional and new. They include imaging and microscopy, cell culture, antibodies, and sophisticated assays. "Progress in genomics will continue to make some gains. Genomics has helped to pinpoint patterns of mutations associated with disease states and the heritability of certain diseases," says Daniel Tusé, vice president of business development for **Predictive Diagnostics**, a wholly owned subsidiary of Large Scale Biology Corporation. "Also important are diagnostic or medical imaging with MRI and CT scans that can suggest clinical interventions. And proteomics has a strong future. It will provide valuable snapshots if relevant biomarkers can be developed."

Christian Kier, marketing manager (confocal laser scanning microscopy) for **Leica Microsystems**, points out two key characteristics of today's tools. "Tools are coming into the mainstream, changing from expert user tools to instruments that everyone must use to play this game," he says. "Also, they have broadened in scope. They are now being used as analytical tools."

Current studies of neurological disease provide plenty of reason for hope in tackling neurological ailments. "Recent advances have made it easier to start on drug discovery," says David Jackson, research area manager for molecular and cellular biology at **Invitrogen**. "An evolving consensus has emerged over the past five years about what cell types participate in neurodegenerative pathology, and a better understanding of the role of various forms of amyloid plaques [the abnormalities in the brain that, along with neurofibrillary tangles, define Alzheimer's]," he adds. "With cell-based and organismal model systems, we're coming to a better understanding of the early toxic process. This improved understanding establishes the disease function of new targets, and stimulates our development of improved HTS assays to find drugs that attack neurodegeneration from these new angles."

Ruyi Hoa, assistant director of protein development at **R&D Systems**, echoes that optimism. "For Alzheimer's, finding that the accumulation of beta amyloid is related to pathology is important," she says. "It has stimulated research on developing inhibitors to the enzymes responsible for producing beta amyloid peptide."

Treatments are also developing. "We're seeing the potential for advance of a lot of disease-modifying therapies," Black says. "New techniques, such as those which detect the presence of amyloid pathol-

Inclusion of companies in this article does not indicate endorsement by either AAAS or *Science*, or by interviewee Marcelle Morrison-Bogorad or the National Institute on Aging. Nor is it meant to imply that their products or services are superior to those of other companies.

ogy in living patients, are helping to detect the disease at earlier stages and to differentiate it from other dementias. This will become increasingly important as we develop treatments to address the underlying disease process, rather than just treating symptoms."

Current Projects and Past History

Several other current projects hold promise for the development of diagnostic methods and treatments for neurological conditions. "On the diagnostic front, people are looking for biomarkers to indicate the disease early," explains Mary Lopez, strategic collaborations leader for molecular medicine business at **PerkinElmer Life and Analytical Sciences.** "Others are working on ways to delay the symptoms. There's also work on the connection between Alzheimer's and diabetes. People are also beginning to talk about Alzheimer's as type 3 diabetes."

German psychiatrist Alois Alzheimer first described the progressive and degenerative disease that attacks the brain and results in impaired thinking, erratic behavior, and memory loss in 1906. But not until 65 years later did active research on the disease began, and it was less than 20 years ago that researchers began developing solid theories on where to focus therapies. The U.S. Food and Drug Administration approved the first drug to treat the disease in 1993.

According to the **World Health Organization**, about 18 million people worldwide suffer from Alzheimer's disease, a figure projected almost to double by 2025. Caring for Alzheimer's patients exerts huge costs. One estimate suggests that the direct and total national cost of dealing with the disease in 2000 reached about $536 billion and $1.75 trillion, respectively. And families and friends who take care of patients experience emotional, physical, and financial stresses that are impossible to quantify.

Scientists have compelling evidence that genetic predisposition underlies the development of the disease. Rare cases, often with an early age of onset, are caused by dominant genes that run in families. Mutations in the amyloid precursor protein gene and presenilin-1 or presenilin-2 genes have been documented in some families. The presenilins are essential components of the proteolytic processing machinery that produces beta amyloid peptides through cleavage of amyloid precursor protein. The disease is definitely linked to the 1st, 14th, or 21st chromosomes. While researchers have identified a gene, ApoE4 on chromosome 19 that predisposes to the most common form of Alzheimer's, late onset disease, it also seems to involve other risks and protective genes, as well as environmental factors.

Tantalizing Results

Morrison-Bogorad sums up the current state of research on Alzheimer's disease. "We've been able to move from looking at the later stages of Alzheimer's to understanding the early pathology," she says. "We have a much better idea of mild cognitive impairment with memory decline, which leads to Alzheimer's in three to eight years after it's diagnosed in most cases. Alzheimer's centers have combined the clinical, pathological, and basic science into one place; interdisciplinary research in these places has generated great excitement. We have also seen very recent evidence that puts the growth factor gene at the center of some dementias. And we have identified such potential risk factors for Alzheimer's as heart disease, diabetes, and even sleep disturbances. We have a number of studies going on in these areas to see if very tantalizing results from animal and epidemiology studies are borne out by clinical trials."

As they set out to follow up on those advances, researchers in the field face several tough challenges. "The major one is identifying the early events that initiate disease and are involved in disease progression. This is crucial for developing therapies," Morrison-Bogorad explains. "Added to that is how you identify people very early as they develop Alzheimer's. You need knowledge of who to treat as well as what to treat with. And third, and increasingly important, is to understand how changes in the brain and body that result from aging contribute to Alzheimer's."

Difficulties abound at both the basic and clinical levels. "You have problems getting a diagnosis that's accurate," Tusé says. "By the time tests show definite signs of Alzheimer's, the disease may be entrenched. And retrieving samples from neural tissue is not something that you can do easily." Richard Eglen, head of R&D reagents at PerkinElmer Life and Analytical Sciences, highlights a problem in the lab. "The first issue is identifying some of the targets for treatment of the disease," he says. "Many of the early receptors have proved disappointing. There's also the difficulty of setting up clinical trials. Patients tend to die before the trials are over."

Microscopes, Cells, and Tissues

The complexity of the human nervous system, which consists of more than a trillion nerve cells, helps to explain such difficulties. Interconnections between these cells complicate the puzzle further. To explore those issues, researchers turn to a traditional tool: the microscope. While once relatively crude and cumbersome, these instruments have morphed over the years into laboratory tools that are easy to use and able to capture highly detailed images of cellular and subcellular components. "Technically, imaging systems have become much more user-friendly and much more reliable," Leica's Kier says.

Other types of microscopy for studying cells and cellular components include phase-contrast, dark-field, and fluorescence microscopy. "Multiphoton imaging is very important for the neurosciences in particular," Kier continues. "And live cell studies and calcium imaging, which involve detecting and understanding very fast events, require the speed of a digital camera or fast confocal scanners." Other major producers of microscopes include **Carl Zeiss**, **Nikon**, and **Olympus**. Like Leica those vendors also offer analytical software for data analysis.

In addition to microscopes, the study of living cells requires cell culture media and reagents. To keep cells in culture **continued ›**

alive and well during experiments to study the response of nerve and other cells to changing environments, researchers and manufacturers have developed growth media and growth factors. Companies that offer those products include **ATCC**, Invitrogen, R&D Systems, and **StemCell Technologies**.

Several vendors also supply cells and tissue for research use. **Asterand** provides human tissue samples for neurological and cancer research, while **Cambrex** offers both cell systems and related products like cell culture media and sera.

The nervous system consists of a number of different cell types, including neurons, astrocytes, oligodendrites, and Schwann cells. Each type of cell possesses unique markers that scientists can use to identify it and separate it from other types. Antibodies to these markers are ideal for differentiating the neural cells. Researchers can also tag antibodies with different labels for multiplexed (simultaneous labeling) experiments.

Applications of Antibodies

Researchers also study antibodies to understand better the role they play in the process and progression of disease. Several scientific teams use antibodies tagged with labels such as fluorescein and other molecules that allow them to identify and locate specific proteins in or on a cell. These antibodies can also find application in histochemical applications, in which sections of the cell fixed in paraffin are stained with antibodies against a specific molecule. Teams can identify the tagged cells using microscopy, fluorescent readers, or flow cytometers. Scientists can also use antibodies generated to a specific protein to "find" that unique protein in a cellular extract. "The antibodies have to be specific, to help recognize the molecules," R&D Systems' Hao explains.

Companies such as **Chemicon**, **Dako**, R&D Systems, and **Upstate** provide antibodies tagged with markers to eliminate the need for conjugating the antibody with a label. "We have monoclonal and polyclonal antibodies; we have more than 6,000 antibodies, raised against 14 different species," Hao says. "Many have multiple applications."

Neurochemicals, certain biochemicals, and bioactive peptides represent more specialized tools applied to neuroscience. Early corporate entrants to the field, such as **Biomol** and **Tocris Cookson**, provided purified biochemicals and reagents specially tested for use in cell signaling research. To complement their reagents, those vendors offer kits for studying apoptosis, protein phosphorylation, and gene regulation.

Cayman Chemical Company and **EMD Biosciences** offer a wide range of kits and reagents for biochemical assays and neuroscience studies. These products combine all the materials needed to study a particular cellular function and eliminate some of the unknowns involved in sourcing different reagents and biochemicals from various providers. The suppliers have use-tested most of their kits to ensure that users can learn quickly how to conduct such experiments.

Many of the drugs originally developed as treatments for disease also have value for basic research in neuroscience. The reason: Those drugs have properties of great interest to laboratory researchers. Indeed, some of the drugs that fail clinical trials turn out to be valuable reagents in basic research. They can, for example, allow scientists to study the function of a particular biomolecule. Several companies acquire drugs of this type from major pharmas and offer them as research reagents. Providers of pharmacologicals include **Axxora**, **BD Biosciences**, and **MP Biomedicals**.

Exploring Living Cells

Most traditional assays require a purified cell extract, which can take several hours to prepare and must be done with great care to avoid altering the intracellular contents of a living cell. Investigators must ensure that they don't degrade or change molecules with the mechanical forces they might use to break open a cell. They must also avoid enzymatic degradation of proteins and nucleic acids via native DNase, RNase and protease molecules.

Several companies have responded to these concerns by creating systems that allow researchers to examine intact living cells in cell-based assays. "Development of these assay systems is very tightly coupled with our understanding of the disease mechanism and disease pathways," Invitrogen's Jackson explains. "Once you have a model, you can develop cellular assays that report on the activity of specific disease targets and pathways. You can work out what the pathways are in your model, and then disease researchers can make sure that what you discovered in the cell-based model also applies to the human disease."

In addition to Invitrogen, companies such as BD Biosciences, **Cellomics**, and PerkinElmer Life Sciences have designed systems that can process large numbers of living cells under relatively natural conditions to examine molecular interactions in cells. These systems expose cells to a compound of interest to see if the compound interacts with the living cells.

"We have developed primarily fluorescence-based assays for discovery," Jackson says. "We've been able to engineer assays incorporating target-specific beta lactamase reporters and membrane potential sensors to address many disease pathways." At present, Invitrogen's assays focus on target-specific assays aimed at many disease areas, including some assays with specific relevance to diseases such as Alzheimer's and Parkinson's. PerkinElmer's UltraView live cell system, meanwhile, "allows us to look at the appropriate response we're measuring," Eglen says. "It's a highly sensitive measurement, so you can see very small changes." continued ›

Look for this upcoming Life Science Technology article

Nanobiotechnology . . . . . 3 November

Or, access past articles at http://www.sciencemag.org/products/articles.dtl

Laboratory Automation . . . . . 13 January
Combinatorial and Computational Chemistry . . . . . 17 March
Cell Signaling in Cancer Research . . . . . 24 March
Proteomics . . . . . 14 April
Biochips and Lab-on-a-Chip Devices . . . . . 5 May
Pharmacogenomics . . . . . 28 July
Mass Spectrometry . . . . . 1 September
Functional Genomics . . . . . 29 September

For news and research with impact, turn to *Science*

There's only one source for news and research with the greatest impact – *Science*. With over 700,000 weekly print readers, and millions more online, *Science* ranks as one of the most highly read multidisciplinary journals in the world. And for impact, *Science* can't be beat. According to the recently released Thomson ISI Journal Citation Report 2005, *Science* ranked as the No. 1 most-cited multidisciplinary journal with a citation factor of 31. Founded in 1880 by inventor Thomas Edison, and published by the nonprofit AAAS, *Science*'s reputation as the leading source for news, research, and leading edge presentation of content continues to grow. Looking for news and research that will impact the world tomorrow? Then look in *Science*.

www.sciencemag.org

To join AAAS and receive your own personal copy of *Science* every week go to **www.aaas.org/join**

Science
AAAS

Testing Biomarkers

An increasingly important theme of neurological studies involves biomarkers. "The tools we are focusing on are for noninvasive testing of biomarkers, through blood and urine," says PerkinElmer's Lopez. "In diseases where diagnosis is a problem, a valid assay using samples obtained by noninvasive means is important," Predictive Diagnostics' Tusé adds. "Samples of blood, saliva, tears, and urine are much more important than others."

The two companies recently used a simple blood test to identify a series of biomarkers that appear to differentiate between individuals with Alzheimer's disease and those without cognitive impairment. PerkinElmer's BioXPRESSION Biomarker platform and Predictive Diagnostics' proprietary Biomarker Amplifier and Filler (BAMF) technology combined to analyze the blood in such a way as to identify patterns of proteins and peptides that distinguish Alzheimer's patients from those without clinical signs.

Both partners have moved on to more advanced methodology. "We have another relationship that we are pursuing more actively with **Nonlinear Dynamics**; we co-developed the mass spectrometry program PG 600 with them. The program allows you to compare rich data sets from mass spectrometry and look for specific markers," Lopez says. Predictive Diagnostics has replaced BAMF with what it calls profile technology. "It consists of machine learning tools combined with algorithms," Tusé explains. "We have set our sights on autoimmune diseases and Alzheimer's, for which we found promising biomarkers in blood samples." In collaborative work with **Harvard Medical School** and **Tufts University**, the company has shown that the profile technology is both robust and highly sensitive.

Drugs against Neurological Diseases

Several pharmaceutical firms, including **GlaxoSmithKline**, **Lexicon Genetics**, and **Pfizer**, have begun to develop drugs to combat Alzheimer's and other neurological diseases. Wyeth has a multiplatform approach that includes small molecule–based, peptide-based, and antibody-based platforms. Drugs under development include gamma-secretase inhibitors that reverse memory deficits in some model animals, PAI-1 inhibitors that reduce plasma and brain levels of beta-amyloid in mouse models, and active immunization programs that alter the progression of disease.

Why such a variety of approaches? "This is a hard target," Black says. "There's a lot of unexplained biology that you have to dig through in preclinical drug development. As we gain more understanding of the genetic causes of the disease and its pathology, we may be better able to tailor our therapies to individual patients with Alzheimer's disease."

Wyeth is also partnering with biotech companies **Curis**, **Elan**, and **Sienabiotech** to speed up the drug discovery and development process. "We acknowledge that our own basic discovery compounds are not the only sources of really exciting compounds," Black says. "We have our homegrown compounds, which we're very proud of, and we've made a partnership with Elan because it's an exciting potential treatment."

What will come next in basic and clinical research on Alzheimer's and other neurological diseases? "Testing of the amyloid hypothesis will come soon," the National Institute on Aging's Morrison-Bogorad says. "We will develop markers for evaluating therapies and drug trials. We'll develop some of the promising translational approaches we're funding. We'll look at amyloid in living patients using positron emission tomography. And we need to work on delivering drugs to the brain through the blood-brain barrier."

Peter Gwynne (pgwynne767@aol.com) is a freelance science writer based on Cape Cod, Massachusetts, U.S.A. Gary Heebner (gheebner@cell-associates.com) is a marketing consultant with Cell Associates in St. Louis, Missouri, U.S.A.

Featured Companies

American Type Culture Collection (ATCC), cell culture media and reagents, http://www.atcc.org

Asterand, Inc., cell and tissue lines, http://www.asterand.com

Axxora, LLC, biochemicals and reagents for cell biology research, http://www.axxora.com

BD Biosciences, biochemicals and reagents for cell biology research, http://www.bd.com

Biomol Research Laboratories, Inc., biochemicals and reagents for cell biology research, http://www.biomol.com

Cambrex Corporation, cell and tissue lines, http://www.cambrex.com

Carl Zeiss, imaging detection systems, microscopes, http://www.zeiss.com/lsm

Cayman Chemical Company, biochemicals and reagents for cell biology research, http://www.caymanchem.com

Cellomics Inc., automated whole cell assay systems, http://www.cellomics.com

Chemicon International (a Serologicals Company), antibodies for cell biology research, http://www.chemicon.com

Curis, Inc., drug development, http://www.curis.com

Dako, antibodies for cell biology research, http://www.dako.com

Elan Corporation, biotechnology, http://www.elan.com

EMD Biosciences (an affiliate of Merck KGaA), biochemicals and reagents for cell biology research, http://www.emdbiosciences.com

GlaxoSmithKline, pharmaceuticals, http://www.us.gsk.com

Harvard Medical School, medical school, http://hms.harvard.edu

Invitrogen Corporation, cell culture media and reagents, http://www.invitrogen.com

Leica Microsystems, imaging detection systems, microscopes, http://www.leica-microsystems.com

Lexicon Genetics, Inc., drug discovery and development, http://www.lexgen.com

MP Biomedicals, biochemicals and reagents for cell biology research, http://www.mpbio.com

Nikon, imaging detection systems, microscopes, http://www.nikonusa.com/microscopes

Nonlinear Dynamics, Ltd., bioinformatics software, http://www.nonlinear.com

Olympus Corporation, imaging detection systems, microscopes, http://www.olympus.com

PerkinElmer Life and Analytical Sciences, automated whole cell assay systems, http://las.perkinelmer.com

Pfizer, Inc., pharmaceuticals, http://www.pfizer.com

Predictive Diagnostics, Inc., biomarker-based diagnostics, http://www.predictivediagnostics.com

R&D Systems, Inc., biochemicals and reagents for cell biology research, http://www.rndsystems.com

Sienabiotech, drug discovery, http://www.sienabiotech.com

StemCell Technologies, cell culture media and reagents, http://www.stemcell.com

Tocris Cookson, Ltd., biochemicals and reagents for cell biology research, http://www.tocris.com

Tufts University, university, http://www.tufts.edu

Upstate, antibodies for cell biology research, http://www.upstate.com

U.S. National Institute on Aging, government research center, http://www.nia.nih.gov

World Health Organization (WHO), international health organization, http://www.who.int

Wyeth Research, pharmaceuticals, http://www.wyeth.com

POSITIONS OPEN

FACULTY POSITION IN CELL PHYSIOLOGY/DEVELOPMENTAL BIOLOGY
Ohio University

The Department of Biomedical Sciences at the Ohio University College of Osteopathic Medicine invites applications for an 11-month appointment as a tenure-track **ASSISTANT/ASSOCIATE PROFESSOR** using cellular and molecular approaches to improve understanding of basic physiology and development, mechanisms of disease or therapy. The appointed individual will be expected to establish and maintain an active, externally funded research program. Collaborations are desirable in the College's areas of strength. Please visit our **website: http://www.oucom.ohiou.edu/dbms/index.htm** for information on areas of research in the Department. The successful candidate will also participate in the delivery of our integrated medical curriculum in area(s) of their expertise.

A Ph.D. (or equivalent) and a minimum of two years of postdoctoral training are required. Salary will be commensurate with experience. To apply, curriculum vitae and statements of research and teaching interests must be submitted online at **website: http://www.ohiouniversityjobs.com/applicants/Central?quickFind=52023.** Also mail three representative papers, and arrange to have three letters of recommendation sent to: **Cell Physiology/Developmental Biology Search Committee Chair, Department of Biomedical Sciences, 228 Irvine Hall, Ohio University, Athens, OH 45701**. For questions about the position, contact **Felicia V. Nowak, M.D., Ph.D.** at **e-mail: nowak@ohiou.edu.** Review of candidates will begin December 1, 2006, but applications will be accepted until the position is filled. We seek a candidate with a commitment to working effectively with students, faculty, and staff from diverse backgrounds. *Ohio University is an Affirmative Action, Equal Opportunity Employer with a dual-career network* ***(website: http://www.ohio.edu/dual).***

UNIVERSITY OF ALABAMA, BIRMINGHAM
School of Dentistry

The School of Dentistry (SOD) Institute of Oral Health Research, University of Alabama, Birmingham (UAB), is seeking full-time research–intensive **FACULTY MEMBERS.** Candidates should possess a D.D.S./D.M.D./M.D. and/or a Ph.D. degree with preferably a documented history of funding and research productivity in the field of craniofacial/developmental biology (tooth, bone, salivary glands, taste, et cetera), genetics, tissue bioengineering or gene therapy. Successful candidates will be expected to establish a major research program within the SOD and develop collaborative research partnerships within the broader UAB research community **(website: http://www.uab.edu).** UAB is a research-intensive Institution receiving over $430 million in funding in fiscal year 2005 with the SOD ranked tenth in National Institute of Dental and Craniofacial Research funding. It has a long history of interdisciplinary research collaborations and resources including the Centers for Arthritis and Musculoskeletal Disease, Biomatrix Engineering and Regenerative Medicine, Gene Therapy, Human Genetic, Metabolic Bone Disease, and Neuroimmunology. Research facilities and startup packages for this appointment will be appropriate for the successful candidate(s). Salary, tenure status, and rank will be commensurate with experience and funding. Review of applications will begin immediately and continue until the position is filled. Please send a letter of interest, curriculum vitae, and a statement of research interest, goals and future directions to: **Mary MacDougall, Associate Dean for Research, School of Dentistry, 1530 3rd Avenue South, SDB 702, Birmingham, AL 35294-0007 U.S.A. (E-mail: macdougall@uab.edu)**

UAB is an Equal Opportunity/Affirmative Action Employer. Women and minorities are encouraged to apply.

POSITIONS OPEN

JAMES A. MOORE CHAIR IN PARKINSON'S RESEARCH
Pacific Parkinson's Research Centre and The University of British Columbia Brain Research Centre Department of Medicine University of British Columbia

The Pacific Parkinson's Research Centre and Brain Research Centre at the University of British Columbia (UBC) are seeking to recruit a researcher in Parkinson's disease. The position is open to either a **CLINICIAN-SCIENTIST** or a **FUNDAMENTAL SCIENTIST.** The successful applicant will have a strong track record of independent research with a focus on Parkinson's disease, as evidenced by peer-reviewed grant support and a strong publication record. The selected individual will be expected to mount an independent research program, but one that is highly interactive with the existing program at the Pacific Parkinson's Research Center, and that will be incorporated into the Center's Canadian Institutes of Health Research Team Grant in Parkinson's disease. Team investigators are studying the etiology of Parkinson's disease, the pathogenesis of long-term complications and disease progression, as well as the relationship between Parkinson's disease and disordered monoaminergic function in the central nervous system.

The successful candidate will be expected to act in a public role for the Centre's research program and to assist relevant foundations in educating the public, persons living with Parkinson's, and their caregivers about the disease and about the research program. Clinician scientists are encouraged to apply; however, the emphasis in selection will be on a strong, innovative research program related to Parkinson's and the ability to interact with an established group.

This is a grant tenure/grant tenure-track position at the rank of **ASSOCIATE or FULL PROFESSOR.** Salary and rank will be commensurate with experience.

The anticipated start date is July 1, 2007.

Please submit a letter of application, curriculum vitae, and a statement of areas of interest, expertise, and strengths and the names of three references no later than November 30, 2006.

Applications should be directed to:

Dr. Jon Stoessl
Professor and Director
Pacific Parkinson's Research Centre
M36 Purdy Pavilion
2221 Wesbrook Mall
Vancouver, BC V6T 2B5
Fax: 604-822-7866
E-mail: jstoessl@interchange.ubc.ca

UBC hires on the basis of merit and is committed to employment equity. We encourage all qualified persons to apply, however, Canadians and permanent residents of Canada will be given priority.

FACULTY POSITION IN SYNTHETIC ORGANIC CHEMISTRY
University of Colorado

The Department of Chemistry and Biochemistry at the University of Colorado, Boulder, invites applications for a Tenured or Tenure-Track position in the area of synthetic organic chemistry. Of particular interest are candidates with research programs in natural products synthesis, or methods development for natural products synthesis. A Ph.D. in chemistry is required. We are seeking applicants who will establish a vigorous and innovative research program and who will contribute to both undergraduate and graduate teaching. Applicants should submit curriculum vitae, undergraduate and graduate transcripts, a description of proposed research, and arrange to have three letters of recommendation sent to: **Professor Tarek Sammakia, Chair, Synthetic Organic Search Committee, Department of Chemistry and Biochemistry, 215 UCB, University of Colorado, Boulder, CO 80309-0215.** Review of applications will begin October 16, 2006, and will continue until the position is filled. *The University of Colorado at Boulder is committed to diversity and equality in education and employment.*

Careers in Stem Cell Research

Rejuvenating Biology and Medicine

Everything in an adult organism comes from stem cells. As scientists learn to better understand and direct the path of these cells, it will enhance basic biological and clinical research. The experts interviewed here see a long future ahead for researchers in these areas. BY MIKE MAY

With all of the excitement during the past few years, stem cells seem like an entirely new realm of biology and medicine. Nonetheless, scientists started studying these cells long ago. Now, though, scientists seem on the verge of turning stem cells into one of science's most powerful tools of all time. In fact, these cells help scientists learn more about basic biology and give physicians new tools against disease. The real power of these cells lies ahead, and there will be plenty of room for trained scientists.

"Embryonic stem cells are a cellular window into pluripotency and infinite replicative potential," says Edison Liu, executive director at the Genome Institute of Singapore. "So the detailed investigation on stem cells will give insight into fate decisions of early embryonic cells." He adds that these cells offer great potential as regenerative therapies.

"It doesn't get mentioned so much," says Beth Donley, executive director of the WiCell Research Institute at the University of Wisconsin, Madison, "but stem cells provide an incredible opportunity to study developmental biology." For these cells, the breadth of application will depend largely on the extent of the scientific imagination. Austin Smith, chair of the Institute for Stem Cell Biology CONTINUED »

Genome Institute of Singapore
http://www.gis.a-star.edu.sg

Lund University
http://www.lu.se/o.o.i.s/450

Massachusetts Institute of Technology
http://web.mit.edu

Newcastle University
http://www.newcastle.ac.uk

University of Cambridge
http://www.cam.ac.uk

University of Washington
www.washington.edu

WiCell Research Institute
http://www.wicell.org

Careers in Stem Cell Research

at the University of Cambridge, says, "Stem cells are used in many different types of research: uncovering basic developmental mechanisms, exploring mechanisms of cell proliferation, and trying to develop cell transplantations and drug screening platforms." He adds, "For me, it's just curiosity driven. I want to understand how these cells work and what they do."

BETH DONLEY

Spreading Stem Cells Around

With stem cells, most people think of using them for cell therapy or regenerative medicine in general. For example, James L. Sherley, associate professor of biological engineering at the Massachusetts Institute of Technology, says, "You could expand adult stem cells, which have long-term division capabilities, and then induce them to make non-stem cell progeny cells that differentiate into mature cell types, which could be used directly or used to make therapeutic biomolecules."

Stem cells could also improve gene therapy. A hematopoetic stem cell, for instance, could be engineered to carry the desired gene. Then, the hematopoetic cells could be put in a person through a bone marrow transplant. The person's body would then grow mature cells that express the gene—one that cures a disease.

Sometimes, scientists can even gather stem cells from unexpected sources, such as umbilical cord blood. Colin McGuckin, professor of regenerative medicine at Newcastle University in the United Kingdom, says, "Umbilical cord blood stem cells give us lots of tissue." His local hospital, for example, delivers 6,000 babies a year. "We have a cord blood bank," McGuckin says, "and we've used that to transplant people who need new bone marrow."

Despite all of the clinical interest in stem cells, basic scientists find them just as appealing. "One of the most exciting areas is the basic biology of how stem cells proliferate and make decisions between self-renewal and differentiation. Developmental biologists have grappled with these issues for a hundred years," says Randall T. Moon, professor of pharmacology, Howard Hughes Medical Institute investigator, and director of the Institute for Stem Cell and Regenerative Medicine at the University of Washington in Seattle.

Visit **www.sciencecareers.org** and plan to attend upcoming meetings and job fairs that will help further your career.

Still, Moon sees beyond the basics. "Tissue engineering is a fairly underutilized area," he says. "Instead of growing cells for transplanting, you could take the cells and entice them to grow over scaffolds into a structure or organ in vitro and then transplant organs and tissues rather than cells into patients." He says that bladders have already been made just that way and used in people.

The therapeutic use of stem cells, though, will apply to many organ systems. Olle Lindvall, professor of neurology at Lund University, started working in neuroscience nearly 30 years ago. He entered this field with an interest in repairing the brain. "From my perspective," says Lindvall, "stem cell research—in the long term—can completely change our possibilities to repair the brain and do something for many neurological patients where we have nothing today."

OLLE LINDVALL

Rules and Regulations

For research purposes, stem cells come under three general categories: human embryonic stem cells, adult human stem cells, and those from other organisms. Using human embryonic stem cells raises the most contention, because they must be collected from an embryo. "There really aren't any issues in doing research with adult stem cells," says Sherley. "They come from an adult donor, and you can get informed consent." Moreover, obtaining these cells causes little if any harm to the donor. Even in cases like aspirating cells from the bone marrow, which carries some risk, many people volunteer to provide cells in this way.

Still, virtually all countries regulate the use of human stem cells, and the rules vary from country to country. "In the United Kingdom," says McGuckin, "we can work on virtually any type of stem cells, but the work we do is very tightly regulated by law." For example, to work on human embryonic stem cells, scientists in the United Kingdom must obtain a license and justify how the cells will be used.

"The diversity of attitudes across Europe," say McGuckin, "is amazing. Some countries are okay with working on all sorts of stem cells, and some countries are not." For example, Poland allows no work on human embryonic stem cells. McGuckin adds, though, that all European countries allow work on stem cells from umbilical cord blood. "With umbilical cord blood," says McGuckin, "it is usually just thrown away."

In Sweden, scientists enjoy an open view about stem cells. "We can do research on human embryonic stem cells, fetal stem cells, and there is also the possibility to use nuclear transfer for therapeutic purposes," Lindvall explains. CONTINUED »

Winning 7 out of 12 Life Science Industry Awards in 2006 was a resounding confirmation of our achievements from the scientific community. Forbes named us to their list of America's best biggest companies and Business 2.0 ranked us in the 100 Fastest Growing Tech Companies. And the list of achievements and accolades goes on.

Proud? You bet! Because behind it all you will find our passionate people collaborating closely to devise the next product or service innovation that will generate scientific breakthroughs around the world. The work is challenging, the rewards are exhilarating and the people are inspiring.

At Invitrogen you will find exceptional career opportunities worldwide in a fast-paced collegial environment, where the potential to learn and grow is virtually unlimited. Our labs and offices are infused with entrepreneurial energy. Yet amidst this dynamic atmosphere all Invitrogen employees share a commitment to integrity and hold fast to the belief that in everything they do, they are making a contribution to a better world. We invite you to join our dedicated team as we continue our quest to improve the human condition.

We have a variety of career opportunities throughout the U.S. and around the world, including:

Business Area Manager – Eugene, OR (Req. 387)

Director, Manufacturing Ops – Madison, WI (Req. 335)

Marketing Manager – Eugene, OR (Req. 441)

Scientist, Transplant Diagnostics – Brown Deere, WI (Req. 442)

Scientist, Epigenetics – Carlsbad, CA (Req. 366)

Sr. Scientist – Eugene, OR (Req. 383)

We invite you to review these and other exceptional position openings at our website where you can apply online.

www.invitrogen.com/careers

We are proud to be an Equal Opportunity Employer dedicated to hiring a diverse work team.

The Life Science LSIA 2006 Industry Awards

B2 100 Fastest-Growing Tech Companies

2006 Forbes THE PLATINUM LIST

invitrogen™

Careers in Stem Cell Research

When it comes to human embryonic stem cells, U.S. scientists with federal funding can only study lines approved by the National Institutes of Health. Nonetheless, scientists can use private or state funding to develop new lines, and some are doing that. In fact, some studies require embryonic stem cells instead of just adult ones. "Research indicates," says Moon, "that adult stem cells do not do everything that you want them to do for developing therapies." For example, embryonic stem cells can make any other type of cell, and adult stem cells cannot.

Donley says that access to human embryonic stem cells is good. She adds that her institute has distributed more than 400 batches of cells to researchers around the world, and the requests are increasing. She says, "Researchers under our agreement can do research in any area and are not restricted in patenting or publishing."

COLIN MCGUCKIN

Training for the Task

Smith believes that only a small number of researchers possess a credible record of work in this field. "Just billing yourself as a stem cell biologist doesn't mean that someone is really in this field," he says. "You must research where you are going to study, if you want to get good training in this field."

Cellular and molecular programs provide a good start, but scientists can come from many other backgrounds. For example, Donley says, "We have scientists who specialize in mass spectrometry, and they do lots of studies on the proteins and growth factors that keep cells undifferentiated or cause them to differentiate."

At the Genome Institute of Singapore, Liu and his colleagues take a genomics approach to understanding the transcriptional regulation of embryonic stem cells. Consequently, scientists there need a background in cellular and developmental biology. Liu adds, "A key skill has become computer analysis. So computational biology is becoming an important facet of the research."

In fact, the road ahead for basic research on stem cells will cover many topics: controlling stem cells, learning how they divide and mature, and creating techniques to influence stem cells to make particular cells. "We need people interested in developmental biology, basic cell biology, and molecular biology," says Lindvall. "That will be very, very important." He adds that scientists skilled in imaging will also be in demand for research on stem cells. "They will help us follow what is happening in the body with these cells," he says. Although Lindvall sees basic research as today's most pressing need, he also believes that clinical and basic scientists should work together to create a roadmap to the clinic.

In addition, research on adult stem cells is expected to grow quickly. "These cells can go into people more easily," says McGuckin. "In America and across Europe, more money is going into adult stem cells, bit by bit." Some examples already exist for using these cells. McGuckin mentions the use of bone marrow stem cells to treat people with a myocardial infarction, where the heart gets starved for blood. "These people need to grow new blood vessels in the heart," says McGuckin, "and bone marrow stem cells have the ability to do that." Moreover, he points out that stem cells from an umbilical cord can treat many diseases right now. He says, "People with leukemia often need radiation and chemotherapy, and then they need a new blood system. Cord blood can give them that." He and his colleagues are also developing a treatment for liver diseases that uses stem cells from cord blood. This work requires a broad scientific team.

Beyond bench skills, Lindvall says that stem cell scientists need a knowledge of ethics. He says, "I ask my new students about their view on the ethical aspects of working on stem cells. If they see none, that is negative from my perspective." He adds, "We need to balance between many aspects."

RANDALL T. MOON

Stem Cell Skills

"You need the same basic skills that any other area of biology requires," says Smith. "You have to be really interested in understanding a fundamental property of the cells." He adds, "A lot of people come with the motivation—that may be laudable—that they may want to cure a particular disease, but then they should be working in a hospital, because the science is not at that stage yet."

Moon believes that working on stem cells goes beyond scientific capabilities. When asked what skills make a young scientist desirable for work in this field, he laughs before saying, "patience." Moon adds, "You need to look at it as an investment in the long haul. There are no quick answers here and no quick therapies." He points out that it took scientists about 15 years to make bone marrow transplants safe and effective. "If you are interested in developing therapies," says Moon, "you need a long-term view that it may take a decade or more for these to emerge." Also, work in this area demands even more than patience. To work toward stem cell therapies, a scientist also needs exposure to bioethics, bioengineering, and technologies like microfluidics and nanotechnology.

Stem cell research also takes a fair amount of specialized training. "Stem cells are extremely difficult to grow," says Moon, "and there's a long learning curve." As a result, Moon believes that scientists in this field must firmly believe that the work can enhance basic CONTINUED »

KECK
SCHOOL OF MEDICINE USC

Cancer Stem Cell Biology

The USC/Norris Comprehensive Cancer Center of the Keck School of Medicine at the University of Southern California seeks applicants for tenure track appointment at the assistant, associate, or full professor level in stem cell biology with a clear relevance to cancer.

Scientists using animal models as well as human stem cells are encouraged to apply.

The USC/Norris Cancer Center offers a highly interactive environment and exciting programs in molecular genetics, epigenetics, gene regulation, tumor microenvironment and cancer immunology, in conjunction with disease-based clinical programs and epidemiology.

The successful applicants will occupy space in the newly constructed Harlyne Norris Research Tower of the USC/Norris Cancer Center, adjacent to the USC Broad Institute for Integrative Biology and Stem Cell Research.

Each applicant should send a current CV, research plan, and 3 letters of reference to:

Dr. Amy Lee
USC/Norris Comprehensive Cancer Center
1441 Eastlake Avenue, MC-9181
Los Angeles, CA 90089-9181.

USC
NORRIS

Tissue Repair & Regeneration

The Center for Stem Cell and Regenerative Medicine (CSCRM) of the Broad Institute for Integrative Biology and Stem Cell Research at the University of Southern California has several positions available at the Assistant and Associate Professor Level for outstanding scientists studying the biology of tissue repair and regeneration, including the stem cell microenvironment.

Applications from individuals working in animal models of tissue regeneration, the interaction of the inflammatory and immune responses with tissue repair, and the stem cell niche, are encouraged.

CSCRM is a new initiative of the Keck School of Medicine at USC that will focus on human embryonic stem cell biology, developmental mechanisms related to stem cell regulation, and the biology of tissue regeneration and repair. There are excellent opportunities for collaboration with other groups active in basic and translational research related to regenerative medicine at the USC Health Sciences campus and the Children's Hospital of Los Angeles.

The CSCRM laboratories have excellent core facilities to support stem cell culture, flow cytometry, confocal microscopy, high throughput cell screening, and genomics.

Individuals interested in applying should contact:

Dr. Martin F. Pera
Director, CSCRM pera@usc.edu

USC is an equal opportunity employer.

Careers in Stem Cell Research

biology and, eventually, benefit patients. He adds, "For anyone who can survive that learning period, I think there will be lots of great jobs."

Despite the specialization, stem cell research requires the basics, as well. Sherley thinks that a young scientist needs a core knowledge of cellular and molecular biology—understanding the lab techniques and the analytical approaches. "Then," he says, "you can bring a lot of other technologies to bear on that core training." For instance, an electrical engineer with a knowledge of biology could develop tools for in vitro or in vivo analysis of stem cells. "There will always be a need for more tools," Sherley says. Without an understanding of cellular and molecular biology, though, Sherley mentions that lots of things can go wrong. "You can spend a lot of time making a device," he says, "and then later find out that it is not effective for the cells."

So thinking ahead never hurts. "This field also demands critical thinking, maybe more so than some others." Sherley says, "There's a lot of misinformation out there, because this field is evolving." So stem cell scientists must read the historical and current literature, process the information, and then come up with the best ways to advance the field.

JAMES L. SHERLEY

An Academic Consensus

When asked where today's stem cell jobs lie, Smith says, "I'd say in all spheres, but not in huge numbers in bioindustry or hospitals." He adds, "In the academic sector, people are crying out for stem cell researchers. In fact, lots of people are moving fields to work on them." Smith also expects the growth in stem cell research to generate more jobs in the future.

The good news is that stem cells cover lots of ground, from molecular biology and biotechnology through cell transplantation and therapy. "It means that people can come into stem cell biology from more or less any field," says Smith. In fact, Smith does not even recruit people who have already worked on stem cells. Instead, he looks for people who could bring expertise from other areas, like neurobiology or proteomics. He says, "You don't have to do your Ph.D. in stem cells to get a postdoc in the area."

Nonetheless, stem cells do not make an easy career. "Like any research, it's bloody hard at times," Smith says, "and just because it's trendy that doesn't make it any easier to do the science." In fact, he thinks that the trendy side might make this field even more difficult. "There is more noise, more bad science," Smith says. "It can be confusing to see the wood from the trees."

In terms of jobs, though, academics looks like the dominant home for stem cell research now and in the near future. "I don't really have any quantitative data," Sherley says, "but my sense—from who I see at meetings and so on—is that the No. 1 source will be university labs." He believes that large pharmas are looking for stem cell scientists to add expertise, but he doesn't see large efforts there yet. On the other hand, he points out that some small biotechnology companies and many government labs do seek stem cell scientists.

AUSTIN SMITH

The United States already possesses hot spots for stem cell research. Moon says that California universities will have the most jobs. He also points to programs at Harvard University, the University of Wisconsin, Madison, and the University of Michigan. Moon's own University of Washington in Seattle just created the Institute for Stem Cell and Regenerative Medicine, which already has 80 labs and is presently constructing a building and receiving private grants in the millions. Moon says, "We are already one of the few places that is successful in raising private money."

The academic trend does not affect the United States alone. Looking at jobs in Sweden, Lindvall says: "It's, of course, academia at the moment. I think currently there is less enthusiasm in industry. There are companies, but as the field develops there will be more business opportunities related to stem cells." He points out that scientists will patent devices—say, for sorting stem cells—that will lead to new business opportunities, as well.

The opportunities in academics, though, are growing. Donley points out that stem cell scientists at the University of Wisconsin, Madison, grew from one in the late 1990s to about 80 today.

A Bright Future

Overall, research on stem cells will grow and generate new opportunities ahead. "I'd say it is probably one of the brightest areas in biology and medicine," says Moon.

From both basic and applied perspectives, stem cells already teach scientists new things at an increasing rate. No one knows how far stem cells can take modern science, but some researchers expect huge changes. "Instead of cutting something out by surgery or treating diseases only with drugs," Moon says, "people may eventually get at least part way to where a starfish has been all along: If it loses an arm, it grows one back. We may not be able to regrow arms, but we can certainly entertain the goal of leveraging knowledge of stem cells to improve treatments for diseases and injuries."

Mike May (mikemay@mindspring.com) is a publishing consultant for science and technology based in the state of Minnesota, U.S.A.

Department of Health and Human Services
National Institutes of Health
National Institute on Aging

Branch Chief, Aging Physiology And Health Scientist Administrator, Cell Structure and Function

The Biology of Aging Program (BAP) in the National Institute on Aging (NIA), a major research component of the National Institutes of Health (NIH) and the Department of Health and Human Services (DHHS), is recruiting for two positions:

Chief, Aging Physiology Branch - preferred expertise in the areas of physiology, endocrinology or other areas of the basic biology of aging. Incumbent will lead a team of Health Science Administrators covering areas of cardiovascular, immunology, musculoskeletal, physiology and endocrinology of aging, including research on stem cell biology and aging. As Branch Chief, will be responsible for overseeing a diverse portfolio of research grants, cooperative agreements and contracts. In addition, serves as Health Science Administrator, with responsibility over one or more grant portfolios within the Branch. In this capacity, the selected candidate will provide scientific and administrative leadership in assisting in the direction and management of a program of research in the area of choice.

Health Scientist Administrator, Genetics and Cell Biology Branch - preferred expertise in the areas of cell and molecular biology or biochemistry. Incumbent will provide scientific and administrative leadership in assisting in the direction and management of a program of research in the area of Cell Biology. The selected candidate will assist in the program development and administration of research grants, training grants, fellowships, cooperative agreements, and contracts dealing with the areas of expertise listed above.

Both positions involve close interaction with scientific investigators, scientific administration of grants and contracts, program planning and development, reporting on scientific progress, and identifying opportunities for future research.

Salary is commensurate with qualifications and research experience (research experience in basic aspects of the biology of aging is highly desirable). For qualifications required, evaluation criteria, and application instructions search for the vacancy announcements at: **http://jobsearch.usajobs.opm.gov/a9nih.asp** - Announcement Numbers: **NIA-07-143796-DE** and **148469-MP** (**Branch Chief**) and **NIA-07-145808-DE** and **NIA-07-148463-MP** (**Health Scientist Administrator**). If additional information is needed, call **Cheryl Caponiti** at **(301) 594-2147**. Applications must be received no later than **November 17, 2006**.

DHHS and NIH are Equal Opportunity Employers.

City of Hope

Where the Power of Knowledge Saves Lives℠

ASSISTANT/ASSOCIATE/FULL PROFESSOR
Islet Stem Cell Biology

As part of its continuing expansion, City of Hope invites applications for an Assistant, Associate or full Professorship in islet stem cell biology, broadly defined to encompass studies of novel sources of islet stem cells as well as their phenotypic commitment and plasticity from the earliest stages. The appointee will have a primary appointment in the Diabetes and Endocrinology program and be part of a growing team of basic, translational and clinical investigators working on cutting-edge strategies for the treatment of diabetes (**http://www.cityofhope.org/Diabetes**). The appointee will also be a member of the Beckman Research Institute that encompasses multiple areas of molecular and cellular biology and may also participate in the institute's Graduate School of Biological Sciences. City of Hope encourages interdisciplinary collaborative interactions and offers strong core resources in transgenic mouse production, functional genomics, oligonucleotide and peptide synthesis, DNA and peptide sequencing, light and electron microscopy, and mass spectrometry/NMR. For further information on research facilities, please visit **http://bricoh.coh.org/**.

Candidates should have a Ph.D. or M.D. degree, postdoctoral experience and the potential to establish, or have already established, an independent research program. Applicants should submit a curriculum vitae, a statement of research interests and plans and at least three letters of reference to: **Islet Stem Cell Biology Search Committee, c/o Susan Kane at City of Hope, 1500 East Duarte Road, Duarte, CA 91010-3000**. E-mail: **facultyrecruit@coh.org**. Closing date for applications is **November 30, 2006**.

City of Hope is an Equal Opportunity Employer.

Faculty Position in Stem Cell Biology
University of California, Riverside

Applications are invited for a position in stem cell biology beginning on or after July 1, 2007. We are particularly interested in outstanding, senior individuals who would be eligible for tenure at the Associate or Full Professor level and who are studying the basic biology and regulation of stem cells and their potential application to human therapy. It is expected that the successful candidate will have strong professional qualifications and an excellent record of research and scholarship. The successful candidate is expected to have or to establish an extramurally funded research program taking advantage of new state opportunities, as well as federal and private sources. The successful candidate will be a member of the UC Riverside stem cell research center, participate in graduate training, and teach in appropriate undergraduate and graduate programs. In addition, the successful applicant will have opportunities for interdisciplinary collaborations with faculty in the life sciences, engineering, and biomedical sciences. Applicants must have a Ph.D., M.D. or equivalent and postdoctoral experience. The position is open at the Assistant, Associate, or Full Professor rank. Senior investigators are particularly encouraged to apply. Rank and salary will be commensurate with experience. Very competitive start-up packages and state-of-the-art facilities are available. UC Riverside is a rapidly growing campus with central proximity to the major biomedical research centers in Southern California.

Applicants should submit a curriculum vitae, a brief statement of research and teaching interests, relevant reprints, and a minimum of 3 letters of recommendation for Assistant Professor applicants; applicants for Associate and full Professor level should provide a minimum of 5 names as extramural referees. Applications can be emailed to **stemcells@ucr.edu**, or alternatively, hardcopy applications can be submitted to **Chair, Stem Cell Search Committee, 1208 Spieth Hall, University of California, Riverside, CA, 92521**. Review of applications will begin **November 1, 2006**. Applications received after this date will be considered until the position is filled.

The University of California is an Equal Opportunity/Affirmative Action Employer.

Cleveland Clinic

The Lerner Research Institute of the Cleveland Clinic Announces the Formation of a New Department of Stem Cell Biology and Regenerative Medicine

We are seeking an established or "emerging leader" stem cell scientist/mammalian developmental biologist to direct our newly created Department. This Department will grow to 8 to 10 faculty and occupy ~25,000 sq. ft. in a new wing of the Lerner Research Institute. The Department will augment strengths in the areas of hematopoietic, cardiovascular, neuroscience and connective tissue stem cell research. The ideal applicant for this position will have a productive research program in fundamental or translational aspects of stem cell and developmental biology and a vision for creating a unique, world-class department. This Department is part of the state-funded Cleveland Center for Stem Cell and Regenerative Medicine involving the Cleveland Clinic, Case Western Reserve University, the Case Medical Center and several corporate partners. The Chair will be provided with a highly competitive, institutionally supported salary, generous start-up funds, and recruitment packages for new faculty.

The Lerner Research Institute with over 140 principal investigators and an annual budget of over $120 million ($83M from NIH) has a commitment to excellence in basic and translational biomedical research.

A letter of interest and curriculum vitae should be e-mailed or sent to:

Paul E. DiCorleto, Ph.D.
Cleveland Clinic Lerner Research Institute, NB21
9500 Euclid Avenue
Cleveland, Ohio 44195
E-mail: dicorlp@ccf.org
http://www.lerner.ccf.org

Ottawa Health Research Institute OHRI IRSO Institut de recherche en santé d'Ottawa

FACULTY POSITIONS IN STEM CELL RESEARCH
Ottawa Health Research Institute
The Ottawa Hospital / University of Ottawa
http//www.ohri.ca

Innovative and creative scientists are invited to apply for faculty positions in the new Centre for Stem Cell Research, adding to an exciting multidisciplinary group of investigators that includes molecular biologists, cell biologists, bioinformaticists and clinician researchers. The newly constructed Centre opening fall of 2006 contains fully equipped laboratories together with genomic, proteomic, imaging, and cell sorting core facilities. Members will hold appointments at the Assistant or Associate Professor level in the Faculty of Medicine at the University of Ottawa. Physician scientists will hold a clinical appointment at the Ottawa Hospital. Central core facilities and resources along with competitive start-up packages will be provided. Stem cell biologists, translational scientists and clinician scientists working with stem cell populations of relevance to regenerative medicine are particularly encouraged to apply.

The successful applicants will be expected to conduct a vigorous program of independent, externally funded research and to teach at the graduate level. Candidates must have a PhD, MD or equivalent degree and postdoctoral experience demonstrating outstanding achievement in their field. Applicants should forward by **December 15, 2006** a CV, copies of representative reprints, statement of research interests, and arrange to have three letters of reference sent to:

Dr. Michael A. Rudnicki, Senior Scientist and Director
Molecular Medicine Program
Ottawa Health Research Institute
501 Smyth Road Ottawa, Ontario
Canada, K1H 8L6

All qualified candidates are encouraged to apply; however, Canadians and permanent residents will be given priority.

Harvard University — Faculty of Arts & Sciences

Director, High Throughput Screening Stem Cell Institute

A new Therapeutic Screening Group at the Harvard Stem Cell Institute (HSCI) is being established to take comprehensive advantage of some of the unique aspects of stem cell biology. The focus will be on establishing novel assays and identifying new drug targets that affect stem cell proliferation and differentiation. The group has a special interest in orphan nervous system disorders, such as Spinal Muscular Atrophy, Amyotrophic Lateral Sclerosis and Huntington's Disease, but anticipates working with other members of the broad HSCI community, including those who have a direct interest in translational research.

We are seeking an individual to head our screening group who has extensive hands-on knowledge of robotics-based screening, particularly in a cell-based, high content format. This individual should have experience with screening small molecule libraries and must be familiar with data analysis and cheminformatics software. A Ph.D. in biological science and 8+ years' related experience, preferably in the industry, are required.

Harvard University is an equal opportunity/affirmative action employer and provides excellent pay and benefits.

For a more detailed description and to submit resume and cover letter, please visit: http://jobs.harvard.edu/jobs/search_req and search using Requisition #27312.

DANA-FARBER CANCER INSTITUTE

POST-DOCTORAL FELLOW

Hematologic Neoplasia

A funded position is available immediately for a qualified post-doctoral fellow in the Department of Hematolgic Neoplasia at the Dana-Farber Cancer Institute, Harvard Medical School. The laboratory focuses upon molecular mechanisms of lymphomagenesis, with a particular focus on multiple myeloma. Emphasis is given to the functional in vitro and in vivo validation of candidate oncogenes and tumor suppressor genes. In vitro studies will include cDNA complementation and shRNAi knock-down technology using lentiviral approaches. In vivo studies involve bone marrow stem cell transplantation and the generation of transgenic/conditional knock-out mice. The molecular and signal transduction aspects of these projects are especially relevant to this work.

Candidates should have obtained a MD or PhD degree, or equivalent, within the last three years. Desired experience include proficiency in manipulating genetic pathways using cDNA overexpression, dominant negatives, and gene knock-downs using RNA, familiarity with lentiviral/retroviral delivery techniques, handling of primary tumor cells, and solid molecular biology and biochemistry skills. Salary is determined according to institutional policy and NIH guidelines. The position will be for a minimum of two years, with additional years possible depending on productivity and interest.

Visit our website www.DFCI.org to apply online, or email recruiting@dfci.harvard.edu. Please reference job req 13977 in the subject line. CVs can be mailed to: Ruben D. Carrasco M.D.,Ph.D, Dana-Farber Cancer Institute, Medical Oncology, Dana 530C, 44 Binney Street, Boston, MA 02115.

HARVARD MEDICAL SCHOOL

The Dana-Farber Cancer Institute is an Equal Opportunity Employer.

SHARE THE VISION. FIND THE CURE

Children's National Medical Center

Director
Translational Epilepsy
Research Program
Children's Research Institute
Children's National Medical Center (CNMC)
Washington, DC

The Center for Neuroscience Research at CNMC is seeking applications for Director of its Translational Epilepsy Research Program. CNMC has strong research programs in neurodevelopment and clinical neurosciences, cancer biology, genetic medicine and neural/muscle degenerative diseases. CNMC is one of the nations leading pediatric research institutions with over $60 million annually in research funding, and is undergoing a major expansion of research space.

The candidate will have: (1) established a nationally/internationally recognized research program in basic/translational epilepsy research; (2) a successful track record in obtaining external funding, (3) the ability to coordinate with superb clinical epilepsy and clinical epilepsy research programs to bring potential therapies to human trials. The position includes a generous package of support and significant space resources, together with a Tenured Associate or Full Professor appointment at the George Washington University School of Medicine.

Applications should be sent by **December 20, 2006** and should include a CV, a letter summarizing qualifications and three references to:

Vittorio Gallo, Ph.D.
Center for Neuroscience Research
Children's National Medical Center, Room 5340
111 Michigan Ave. NW
Washington, DC 20010

Medical College of Georgia 1828

Eminent Scholar
Endowed Professorship in Neuroscience

The Medical College of Georgia (MCG), Georgia's premiere Health Sciences University, is recruiting a recognized senior scientist with national and international stature in Neuroscience for an endowed professorship. Preference will be given to a candidate with a research program that has clear translational relevance and applications, but applicants from all areas of Neuroscience will be considered. Candidates should have a proven track record of well-funded and productive research. The chosen candidate will receive a competitive startup package, along with the endowed professorship. MCG ranks in the top ten public medical schools in basic science grant earnings per faculty member, and current research strengths include stroke, behavioral sciences, learning and cognition, neuropharmacology, epilepsy, neurodegenerative diseases, neural repair, neural signaling, neural stem cell biology, receptor and channel function, synaptic structure, development and plasticity, taste, and vision. Excellent core facilities include Electron Microscopy, Transgenic and Knockout Mouse, Transgenic Zebrafish, Flow Cytometry, Proteomics and Genomics, Small Animal Behavioral Core, Imaging facilities, and a Human Brain lab.

Additional information is available at **www.mcg.edu**, or from the Chair of the search committee **Dr. Darrell Brann** (**dbrann@mcg.edu**). Applications with a statement of research and teaching interests, curriculum vitae, and names and contact information for three references should be sent to: **Ann Gambill, MCG, IMMAG, 1120 15th Street, CB-2803, Augusta, GA 30912** (**agambill@mcg.edu**). Review of applications will begin on **15 November 2006**.

MCG is an EEO/AA/Equal Access Employer. We invite applications or nominations from women, underrepresented racial minorities, and the handicapped.

School of Medicine UC Davis

THE DEPARTMENT OF MEDICAL PATHOLOGY
UNIVERSITY OF CALIFORNIA, DAVIS
Stem Cell Pathologist

The Department of Medical Pathology and Laboratory Medicine in the School of Medicine at the University of California, Davis, announces the search for a full-time academic pathologist to provide prominent leadership in the establishment and development of a basic research program in Stem Cell Research. This recruitment will form a part of the Regenerative Medicine and Stem Cell Program initiative at UC Davis. The successful candidate will have funded independent research programs in an area related to the regulation of stem cell proliferation and differentiation or to the isolation, characterization, and or utilization of stem cells. Emphasis either on basic biological questions or on translational applications is appropriate. Candidates working in any area of stem cell research are encouraged to apply although special consideration will be given to applicants working in the areas of cancer, vascular or hematopoietic stem cells. Candidates at all levels will be considered but special consideration will be given to those at the mid-career level (Associate or Full Professor or equivalent). This position includes an FTE in the School of Medicine, competitive start-up funds, office space, and research space. Access to a large number of core facilities at the University and the UC Davis Medical Center will be available. The successful candidate should possess a M.D., M.D./Ph.D., or Ph.D. degree. Ability to work cooperatively and collegially within a diverse environment is essential.

For full consideration, applications must be received by **November 30, 2006**. However the position will remain open until filled but no later than February 28, 2007. Please forward (1) letter of interest describing research and teaching background; (2) curriculum vitae including reprints of three selected recent publications; and (3) five references (including name, address and phone number and email) to: **Department of Medical Pathology and Laboratory Medicine, University of California, Davis Medical Center, 4400 V Street, PATH Building, Sacramento, CA 95817, Attn: Amanda Scott, Academic Personnel Coordinator**.

The University of California, Davis, is an Affirmative Action/ Equal Opportunity Employer with a strong institutional commitment to the achievement of diversity.

CAREERS IN NEUROSCIENCE

Washington University in St. Louis

ASSISTANT PROFESSOR IN NEUROSCIENCE
Neuroscience Faculty Position:
Department of Biology

The Department of Biology at Washington University in St. Louis seeks a junior colleague at the rank of tenure-track Assistant Professor in the area of Neuroscience. Under exceptional circumstances, tenured appointments may be considered at the rank of associate or full professor. The successful candidate will establish a vigorous research program, and participate in undergraduate and graduate teaching. Candidates with expertise in systems, developmental, computational, or cellular neuroscience, or neuroethology will be viewed with particular interest. All candidates must have their Ph.D. in hand at the time of the appointment. The successful candidate will be encouraged to participate in University-wide initiatives in Neuroscience, Imaging and Systems Biology. For further information, see the Department of Biology (**biology.wustl.edu**) and the Neuroscience Program (**neuroscience.wustl.edu**).

Review of applications will begin **November 1, 2006**. Applications will be accepted until the position is filled. Please submit a cover letter, curriculum vitae, brief statements of research and teaching interests, reprints of up to three papers, and the names and affiliations of three persons who have been asked to send letters of recommendation. We prefer electronic submissions emailed to: **neurosearch@biology.wustl.edu**.

If you prefer hard copies, please send them to: **Chair of Neuroscience Search, Department of Biology, Washington University, Campus Box 1137, One Brookings Drive, St. Louis, MO 63130- 4899**.

Washington University is committed to excellence through diversity, and we particularly encourage applications from persons from underrepresented groups. Washington University is an Affirmative Action Employer.

Georgia State University

Neuroscience Positions at Georgia State University

Georgia State University (**www.gsu.edu**) invites applications for multiple tenure-track faculty positions at all ranks. These positions are part of a major initiative to enhance existing strengths in neuroscience at GSU over the next three years. The GSU neuroscience initiative is coordinated by the Brains and Behavior Program at GSU and the Center for Behavioral Neuroscience. The Brains & Behavior Program is a University Area of Focus that sponsors collaborative research and instruction in all areas of neuroscience across ten Departments (see **http://brainsbehavior.gsu.edu**). The Center for Behavioral Neuroscience is a multi-institutional NSF Science and Technology Center for neuroscience research and teaching across seven Atlanta institutions of higher education (see **www.cbn-atl.org**).

Outstanding applicants from all areas of neuroscience are welcome, although preference will be given to applicants working in the following areas (listed alphabetically):

Behavioral neuroscience and neuroendocrinology of social behavior: Multidisciplinary research investigating the neural systems underlying social behavior and the fundamental neural and hormonal processes that support it, using either standard laboratory animal models or other species (vertebrate or invertebrate) that reflect a neuroethological perspective.

Behavioral neuroscience in nonhuman primates: Research on comparative cognition, social behavior, communication, or higher-order cortical processing in nonhuman primates with a neuroscience or neuroendocrine focus. Primarily behavioral approaches will be considered provided the work has a direct connection to brain function. Research that complements current work at the Georgia State University's Language Research Center (**http://www.gsu.edu/lrc**) is especially attractive.

Cell and molecular neuroscience: Molecular, biochemical, and biophysical processes that underlie sensory, neuronal, synaptic, and effector properties.

Circuit and systems neuroscience: Multidisciplinary experimental approaches to basic neuroscience questions at cellular, circuit or systems levels in vertebrate or invertebrate animals.

Computational neuroscience: Computational, mathematical, or physical simulation methods used to address basic neuroscience questions at the cellular, circuit, systems, and behavioral levels or the development of neuroinformatic, database tools for neuroscience.

Developmental neuroscience: Neural development and other forms of plasticity, including adult neurogenesis.

Human cognitive neuroscience: The neural mechanisms of social cognition or emotional processing in particular, although all areas of human neuroscience will be considered provided they focus on basic mechanisms of neural structure and function in the human brain.

We particularly encourage applications from individuals who use a variety of approaches and who span two or more of these categories. Successful applicants will be individuals who are prepared to take advantage of the interdisciplinary collaborative research opportunities available within the Brains & Behavior Program and, where appropriate, the Center for Behavioral Neuroscience.

Applicants must have a Ph.D. degree with relevant postdoctoral experience and demonstrated ability to conduct an independent research program using modern techniques. They must also have skills and interest in teaching. Submit curriculum vitae, bibliography, a brief statement of professional goals and research interests, and the names and contact information for 3 references either electronically to: **Ms. Tara Alexander at biotea@langate.gsu.edu, with the subject line "Neuroscience Search", or by mail to Brains and Behavior Program, Attn. Ms. Tara Alexander, Georgia State University, P.O. Box 4010, Atlanta, GA 30302-4010, USA**. The review of applications will begin **November 15, 2006** and will continue until positions are filled.

Georgia State University is an Affirmative Action/ Equal Opportunity Employer.

FAU FLORIDA ATLANTIC UNIVERSITY

PH.D. PROGRAM IN COMPLEX SYSTEMS AND BRAIN SCIENCES AT FLORIDA ATLANTIC UNIVERSITY

NIMH PREDOCTORAL FELLOWSHIPS, RESEARCH AND TEACHING ASSISTANTSHIPS

The aim of this program is to train scientists who are both mathematically and biologically literate so that they can fully participate in multidisciplinary research to bring new ways of thinking into neuroscience. Individuals with undergraduate degrees in any pertinent discipline are invited to apply for this 5-year training program at the FAU Center for Complex Systems and Brain Sciences.

Graduate training consists of a core curriculum in nonlinear dynamics, neuroscience, computational modeling and cognitive science. Research areas include sensorimotor coordination and learning, human brain imaging, including functional magnetic resonance imaging, EEG, brainstem mechanisms of behavior, neural growth and development, ion channel dynamics, speech production and perception, neurolinguistics, visual perception, music perception and mathematics of complex systems.

Applicants should complete the application package that can be found on our website **http://www.ccs.fau.edu** and send it together with a letter of interest, vitae and 3 letters of reference to: **Rhona Frankel, Center for Complex Systems and Brain Sciences, BS-12, Florida Atlantic University, 777 Glades Road, Boca Raton, FL 33431**. E-mail: **frankel@ccs.fau.edu**. Deadline: **January 16, 2007**. Additional mandatory FAU application can be found at **http://graduate.fau.edu/GradApp/**.

ANNOUNCEMENTS

Dystonia Medical Research Foundation
1 E. Wacker Drive
Suite 2430
Chicago, IL 60601
312.755.0198

2007 RESEARCH FUNDING OPPORTUNITIES

The **Dystonia Medical Research Foundation** is soliciting grant and fellowship applications for research related to the causes, mechanisms, prevention and treatment of all forms of dystonia, the third most common neurological movement disorder.

Applications are due **January 10, 2007**.

For more information, visit the Foundation website at **www.dystonia-foundation.org**

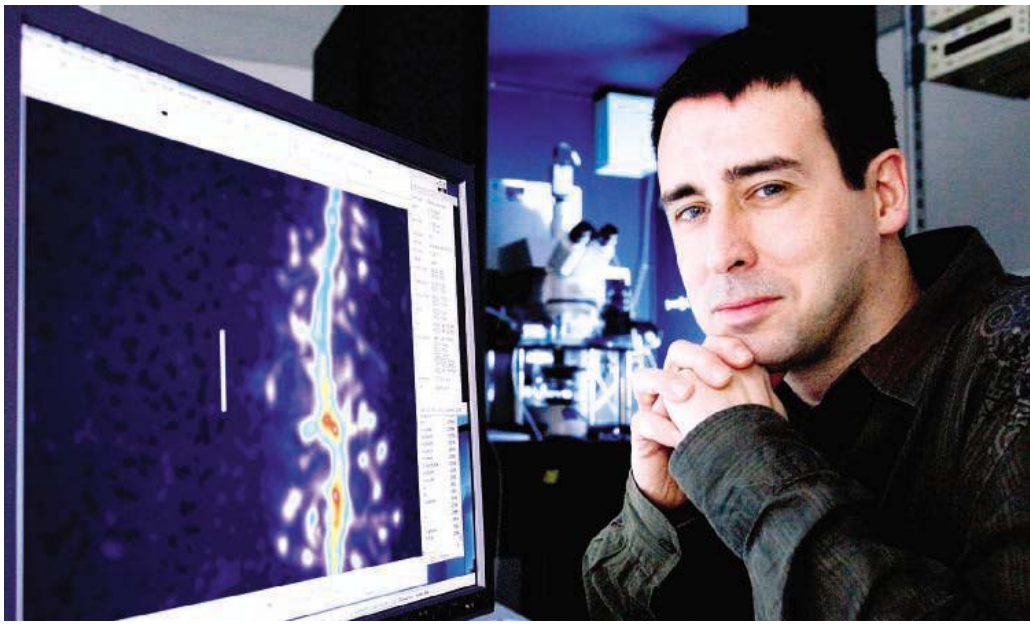
Join one of the world's most exciting neuroscience research teams at the Queensland Brain Institute in Australia

Neuroscience is entering an era of accelerated discovery, promising a new understanding of the mechanisms that regulate brain function and applying this to developing new therapeutics for brain diseases. With this aim, The Queensland Brain Institute (QBI) – part of The University of Queensland – is recruiting up to six new Faculty in the areas of cellular and synaptic plasticity, visual, cognitive and computational neurosciences. The foundation Director of QBI, Professor Perry Bartlett FAA, heads a dynamic Faculty of senior research neuroscientists which includes Pankaj Sah, Mandyam Srinivasan FAA, FRS, Brent Reynolds, Jason Mattingley, Geoffery Goodhill, Linda Richards and Helen Cooper.

Established in 2003, QBI is home to world-leading research in cellular and synaptic plasticity. Leadership direction builds on the key discovery made by several Faculty members in 1992 that the adult brain contains stem cells capable of producing new neurons. QBI is exploring ways to stimulate the production of new functional nerve cells to overcome diseases such as dementia, stroke and motor neuron disease and to promote optimal brain function.

QBI's research focus is on discovering and understanding the molecular and physiological regulation of brain function. Specifically, QBI targets brain plasticity with six main research themes, all of which will be expanded, in terms of infrastructure and research capacity, over the next five years:

- Cellular plasticity
- Synaptic plasticity
- Cognitive and behavioural neuroscience
- Visual neuroscience
- Mental and neurological disorders
- Computational neuroscience



Above: QBI Research Fellow, Dr John Power

World-class facilities

The Institute's potential to make key advances will be further progressed by the development of a $64 million facility, fitted with state-of-the-art research equipment, and have the capacity to accommodate some 250 scientists and support staff.

Scheduled for completion in July 2007, the new complex will see the Institute evolve into one of the finest neuroscience research centres in the world. QBI's scientific team will also develop leadership in core technology, especially in advanced imaging. The Institute has already installed a16.4Tesla animal MRI and 4T human MRI. In addition, QBI has the world's largest cell-sorting facility dedicated to neuroscience. The new Institute will also have extensive capabilities in animal and human behavioural testing and has developed strong interdisciplinary teams in the area of applying nanotechnology to neuroscience.

Asia–Pacific focus

By developing a highly collaborative network based on 'real' scientific exchanges and joint programs, QBI intends to become a focus for the Asia–Pacific neuroscience community.

Neuroscientists at all levels – up to full professor – are encouraged to apply.

Enquiries to:

Professor Perry Bartlett
p.bartlett@uq.edu.au
www.qbi.uq.edu.au

The University Of Queensland AUSTRALIA | qbi queensland brain institute

Faculty Positions in Molecular Neuroscience
Stark Neurosciences Research Institute
Department of Biochemistry and Molecular Biology
Department of Pharmacology and Toxicology

INDIANA UNIVERSITY SCHOOL OF MEDICINE

The Paul and Carole Stark Neurosciences Research Institute (SNRI) of the Indiana University School of Medicine in Indianapolis announces a search for three new faculty in the area of molecular mechanisms underlying nervous system plasticity as part of a major expansion of neuroscience research over the next several years. The SNRI, in partnership with the Departments of Biochemistry and Molecular Biology and of Pharmacology and Toxicology, seeks **three** outstanding individuals for faculty positions at the **Assistant or Associate Professor** level. More senior individuals with exceptional credentials will be considered. A Ph.D. and/or M.D. degree and at least 3 years of postdoctoral research experience are required, and strong evidence of productivity and grant support are desirable. Of primary interest are candidates who employ pharmacological, molecular, genomic/proteomic and/or chemical approaches to the study of signaling networks underlying synaptic plasticity in the following areas: mechanisms of substance abuse and addiction, neuropsychiatric disorders, or nervous system repair and regeneration. Competitive start-up packages include ample space in a new research building and access to exceptional core research facilities. Primary departmental affiliation will reside in one of the partner departments to be negotiated by mutual interest. Successful candidates will be expected to develop strong extramurally supported research programs, contribute to an already strong, collaborative research environment, and to excel in mentoring graduate and postgraduate trainees. More information about the SNRI and partner departments can be found on our websites (**snri.iusm.iu.edu**, **www.biochemistry.iu.edu**, and **pharmtox.iusm.iu.edu**).

Interested individuals should send a curriculum vitae, a research prospectus, and the names and addresses of 3 references. Application materials will only be accepted in electronic format by submission to the attention of **Dr. Gerry Oxford**, Executive Director, SNRI, IU School of Medicine at **snri@iupui.edu**.

IU is an Equal Employment Opportunity/Affirmative Action Employer, M/F/D.

COLUMBIA UNIVERSITY
IN THE CITY OF NEW YORK

Neuroscience Faculty Recruitment

The Neuroscience Initiative at Columbia University is recruiting faculty with interests in the analysis of neural circuitry through molecular, genetic, cellular electrophysiological, and/or imaging approaches. We are keen to attract individuals whose research program explores neural circuits in genetically tractable model systems and in the context of well-defined behaviors. We encourage applications for positions at the Assistant Professor level but will also consider applications from more senior investigators for positions at the level of Associate or full Professor.

Columbia University currently has a world-renowned program in neurobiology and behavior, and the Neuroscience Initiative aims to enhance interactions between basic and clinical neurosciences and link the neurosciences to other scientific disciplines within the University. Faculty will be affiliated with the Center for Neurobiology and Behavior, and there will be opportunities for strong ties with scientific departments and programs on the Morningside Heights campus.

Applications for this round of recruitment are requested by November 17, 2006. A CV, cover letter including statement of interests, and three letters of reference under separate cover should be e-mailed care of Dr. Sarah Caddick, dgl2102@columbia.edu. In addition, please mail a hard copy of these documents to:

Chair, Neuroscience Search Committee
c/o: Dr. Sarah Caddick
Columbia University
Hammer Health Sciences Center
Room 2-205G
701 West 168th Street
New York NY 10032

Columbia University takes affirmative action to ensure equal employment opportunity.

UF UNIVERSITY of FLORIDA
The Foundation for The Gator Nation

University of Florida McKnight Brain Institute and College of Medicine
Faculty Positions in Neuro-Oncology Research Program

The Mcknight Brain Institute (UFMBI) is expanding its research program in neuro-oncology and invites applications for several tenure-track faculty positions at the rank of assistant, associate or full professor for individuals having a Ph.D. and/or M.D. degree. The program is multidisciplinary in its approach to expanding our basic understanding of brain tumors and the development of new treatments. Support for the program includes state, institutional and endowed sources. The UFMBI serves as an umbrella for the neuro-oncology program that includes the University of Florida Shands Cancer Center, Florida Center for Brain Tumor Research, Preston Wells Brain Tumor Therapy Center and several basic science and clinical departments. A primary academic appointment will be made in one of the affiliated departments in the College of Medicine. At the assistant professor level, candidates will be expected to show the potential to develop an independent but interactive research program whereas at the associate and full professor levels, candidates should have demonstrated excellence in cancer research. Investigators in all areas of brain tumor research such as angiogenesis, cell cycle control, cell signaling, immune-tumor cell interactions, stem cell biology, tumor imaging and mechanistic approaches to novel brain tumor treatments, are encouraged to apply. A commitment to the education of professional and graduate students is expected. The positions offer a competitive salary, a generous start-up package and excellent research facilities.

Applicants should send their curriculum vitae, a description of research interests, and three letters of recommendation to: **Dr. Jeffrey K. Harrison, c/o The Evelyn F. and William L. McKnight Brain Institute of the University of Florida, College of Medicine, P.O. Box 100015, Gainesville FL 32610**. E-mail at **Sabrina.mclaughlin@mbi.ufl.edu**. Application deadline is **November 1, 2006** with an anticipated start date on or after March 1, 2007.

The University of Florida is an Equal Opportunity Employer, and minorities and women are encouraged to apply.

Neurophysiology of Cognition and Neurological Disorders
Gladstone/UCSF Faculty Position

The Gladstone Institute of Neurological Disease and the University of California, San Francisco (UCSF) invite applications for a faculty position at the level of Assistant Investigator/Assistant Professor. Of particular interest are candidates with broad expertise in electrophysiology and chemistry who are interested in investigating experimental models of Alzheimer's disease and other neurological conditions at the molecular, cellular, and systems level. Primary criteria for appointment will be outstanding records of innovative research and academic performance, including landmark papers in leading journals, as well as high potential for establishing a vigorous independent research program, inspiring mentorship, and fruitful collaboration.

The successful candidate will join an interactive group of investigators in Gladstone's state-of-the-art research facility at UCSF's new Mission Bay campus. S/he will have joint appointments in the Gladstone Institute of Neurological Disease, and in the Department of Physiology, the Department of Neurology, and the Neuroscience Program at UCSF. Excellent salary support is provided. Women and minorities are especially encouraged to apply. The search will continue until the position is filled. To ensure full consideration, however, applications should be received by **December 15, 2006**. For additional information on research programs and facilities, see **www.gladstone.ucsf.edu/gind**.

Please send curriculum vitae, description of achievements and research interests, and the names of three references to:

GIND/UCSF Search Committee
1650 Owens Street
San Francisco, CA 94158
gindsearch@gladstone.ucsf.edu
Job # M2227

The J. David Gladstone Institutes and UCSF are Affirmative Action/Equal Opportunity Employers. They undertake affirmative action to assure equal employment opportunity for underutilized minorities and women, for persons with disabilities, and for Vietnam-era veterans and special disabled veterans.

CAREERS IN NEUROSCIENCE

Department of Health and Human Services
National Institutes of Health
National Institute of Allergy and Infectious Diseases

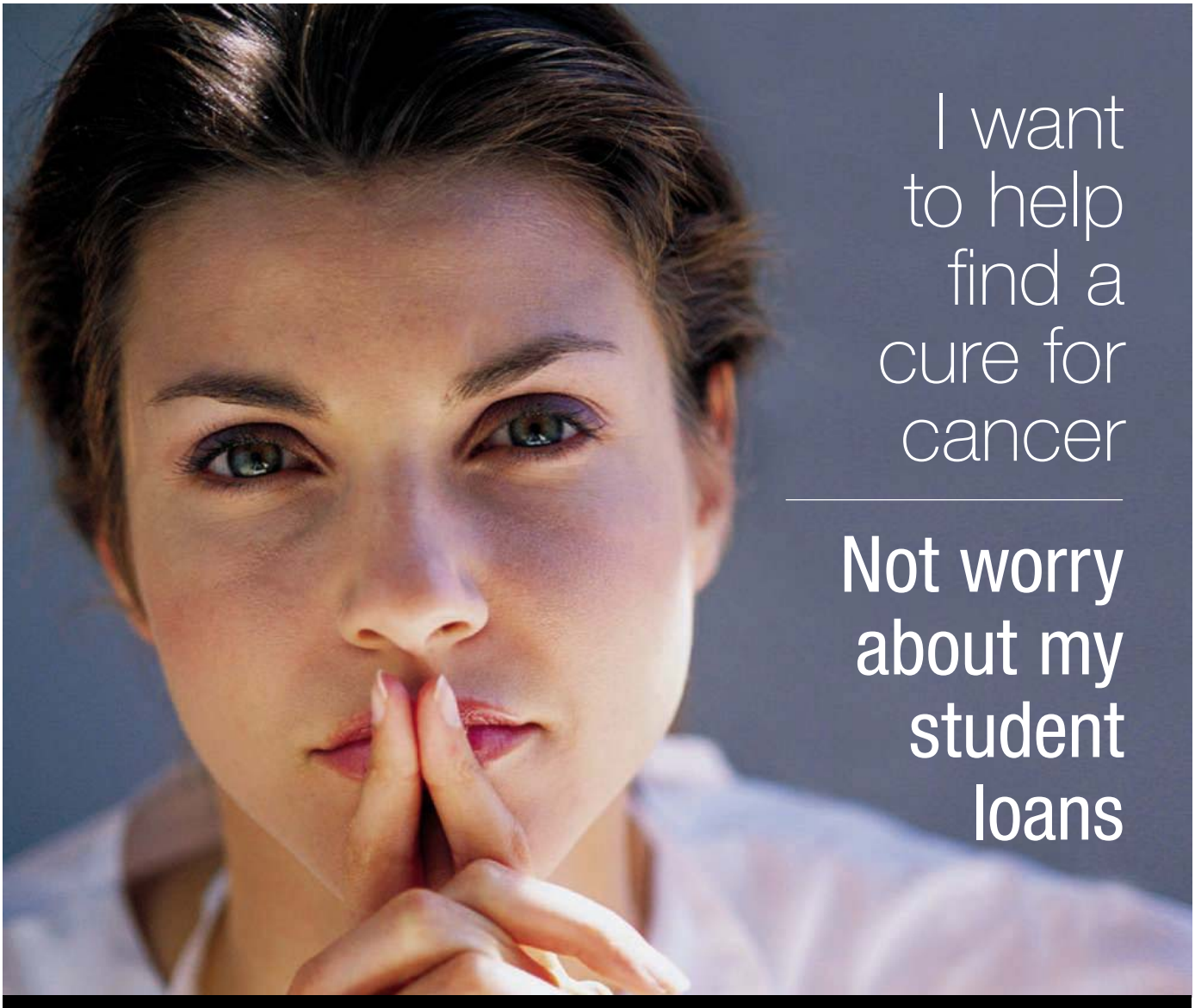
With nation-wide responsibility for improving the health and well being of all Americans, the Department of Health and Human Services oversees the biomedical research programs of the National Institutes of Health (NIH) and those of NIH's research Institutes.

The National Institute of Allergy and Infectious Diseases (NIAID), a major research component of the NIH and the Department of Health and Human Services, is recruiting for a Tenure/Tenure Track position in the Laboratory of Host Defenses (LHD). The LHD studies immune functions essential for host defense against infection (inherited immune deficiencies) and those required for immune homeostasis (autoimmunity associated with excessive inflammation). The LHD seeks an M.D. or M.D., Ph.D. physician scientist to develop an independent translational research program related to the genetic basis, pathophysiology, diagnosis and treatment of autoimmune diseases associated with excessive inflammation. An emphasis on clinical aspects of innate immunity including phagocytic cells, natural killer cells, dendritic cells and other antigen presenting cells, toll-like receptors or other pattern recognition receptors in its interface with acquired immunity is desirable. The applicant should have a strong track record of basic research of the genetic basis of disease and alterations in signaling pathways responsible for immune dysregulation. The applicant must possess expertise and experience in the design and conduct of diagnostic and therapeutic clinical trials studying and treating autoimmune diseases. Strong clinical credentials in a specialty area relevant to the proposed translational research program (relevant specialties include but are not limited to rheumatology, pulmonary diseases, hematology, immunology or infectious diseases) are required. The program of study proposed by the applicant must include both laboratory components and the conduct of clinical protocols to assess new diagnostic and therapeutic modalities to diagnose and treat autoimmunity associated with excessive inflammation. Applicants particularly suitable for this program are those who have knowledge and experience in the development and clinical application of novel biological agents including chemokines, soluble chemokine receptors, adenosine receptor agonists, monoclonal antibodies, cellular therapies including transplantation or gene therapy to correct the abnormalities in immunity, that achieve immune tolerance or to reduce abnormal inflammation.

The applicant must provide evidence in the submitted materials that the applicant has a current license to practice medicine in one of the states of the United States or must have all the credentials required by the State of Maryland for licensing to allow the practice of medicine. These credentials must include but are not limited to having a Doctor of Medicine or Doctor of Osteopathy degree from an accredited school in the U.S. or Canada, or a Doctor of Medicine or equivalent degree from a foreign medical school that provided education and medical knowledge substantially equivalent to accredited schools in the U.S. as demonstrated by permanent certification by the Educational Commission for Foreign Medical Graduates (ECFMG).

To be considered for this position, you will need to submit a curriculum vitae, bibliography, three (3) letters of reference, a detailed statement of research interests, and a hardcopy of selected publications to **Thomas A. Fleisher, MD, Chairperson, NIAID Search Committee, c/o Ms. Anissa N. Hunter, DIR Committee Coordinator, Reference Ad #009, 10 Center Drive MSC 1356, Building 10, Rm. 4A26, Bethesda, Maryland 20892-1356**. Completed applications **MUST** be received by **Thursday, November 15, 2006**. For additional information on this position, and for instructions on submitting your application, please see our website at: **www.niaid.nih.gov**.

WWW.NIH.GOV



I want to help find a cure for cancer

Not worry about my student loans

If you're interested in a biomedical research career, you should know that the National Institutes of Health Loan Repayment Programs may repay up to $35,000 per year of your qualified educational loan debt.

Deadline for Applications is December 1

For more information, visit www.lrp.nih.gov or call 1–866–849–4047

NATIONAL INSTITUTES OF HEALTH

DEPARTMENT OF HEALTH & HUMAN SERVICES · USA

The NIH Loan Repayment Programs | ***Healthier Futures Through Research***

The National Institute of Allergy and Infectious Diseases, a major research component of the NIH and the Department of Health and Human Services, is recruiting a Staff Scientist. The position will be available in the Respiratory Viruses Section of the Laboratory of Infectious Diseases, and scientists with a M.D., D.V.M, or Ph.D. are eligible. The research activity involves (1) examination of the pathogenesis of pandemic and potential pandemic strains of influenza and their evaluation in vitro and in experimental animals; (2) influenza viral genomics, and examination of viral evolution in fitness and host adaptation; and (3) the development of influenza clinical trials in humans. This full-time research position offers a unique opportunity to work on investigations that range from basic molecular biology to clinical research. Staff Scientist applicants should have at least six years of laboratory work experience in molecular and classical virology research; the salary range is $73,178 - $165,195. Preference will be given to candidates who have experience working with avian influenza viruses and those with BSL3 experience. Applicants should submit their curriculum vitae, a letter of research interests, and names and addresses of three references to:**Jeffery K. Taubenberger, MD, PhD, Attn: D. Kyle, NIAID, NIH, Bldg 50 Room 6234, MSC 8007, 50 South Drive, Bethesda, MD 20892-8007, FAX: (301) 496-8312, email: dkyle@niaid.nih.gov**

Review of applicants will begin on **October 30, 2006** and continue until a successful candidate is identified.

The National Institute of Allergy and Infectious Diseases, a major research component of the NIH and the Department of Health and Human Services, is recruiting a Staff Scientist. The position will be available in the Respiratory Viruses Section of the Laboratory of Infectious Diseases, and scientists with a M.D., D.V.M, or Ph.D. are eligible. The research activity involves (1) the development of live attenuated vaccines against potential pandemic strains of influenza and their evaluation in experimental animals as well as in clinical trials in humans; (2) examination of the pathogenesis of avian influenza viruses and SARS-coronavirus; (3) the evaluation of the immunologic determinants of resistance to infection and disease caused by influenza viruses and SARS-coronavirus. This full-time research position offers a unique opportunity to work on investigations that range from basic molecular biology to applied vaccinology. Staff Scientist applicants should have at least six years of laboratory work experience in molecular virology and vaccine research; the salary range is $73,178 - $165,195. Preference will be given to candidates who have experience working with avian influenza viruses. Applicants should submit their curriculum vitae, a letter of research interests, and names and addresses of three references to: **Kanta Subbarao, MD, MPH, Attn: A. LeCointe, NIAID, NIH, Bldg 50 Room 6234, MSC 8007, 50 South Drive, Bethesda, MD 20892-8007, FAX: (301) 496-8312, email: lecointe@niaid.nih.gov**

Review of applicants will begin on November 1, **200**6 and continue until a successful candidate is identified.

Tenure-Track Investigator Position in Immunology and Related Fields

The National Institute of Allergy and Infectious Diseases (NIAID), Division of Intramural Research (DIR) is recruiting for a Tenure-Track Investigator in the Laboratory of Cellular and Molecular Immunology (LCMI). The NIAID is a major research component of the NIH and the Department of Health and Human Services (DHHS).

The Laboratory of Cellular and Molecular Immunology (LCMI) is seeking an M.D., Ph.D., D.V.M., or an equivalent degree for a tenure track position. Candidates with a strong record of creative, scientific accomplishments, and those with a novel, progressive approach to the discipline are particularly encouraged to apply.

The successful candidate will have a unique opportunity to establish an independent research program at the NIH main campus in Bethesda, Maryland. This facility houses one of the largest immunological research communities in the world, with access to flow cytometry, confocal microscopy, mass spectrometry and microarray production. This position will have committed resources for space, a technician and two postdoctoral fellows, as well as an allocated budget to cover service, supplies, animals and salaries.

Salary will be commensurate with research experience and accomplishments. A full Civil Service package of benefits is available, including retirement, health, life, long term insurance care and Thrift Savings Plan.

Address any questions about this position to **Dr. Ron Schwartz at rs34r@nih.gov**. Please note search #008 in all correspondence and when sending materials. To apply, candidates must submit: curriculum vitae and bibliography, and a 2-3 page description of a proposed research program preferably via email to **Ms. Felicia Braunstein at braunsteinf@niaid.nih.gov**. In addition, three letters of recommendation must be sent to **Ms. Felicia Braunstein, Committee Manager, NIAID, NIH; Bldg. 10, Rm.4A30, MSC-1349; Bethesda, MD 20892-1349**. All applications must be received by **November 27, 2006**. All applicants will be notified by e-mail or phone when their applications are received and then complete.

DEPARTMENT OF HEALTH AND HUMAN SERVICES
NATIONAL INSTITUTES OF HEALTH
OFFICE OF THE DIRECTOR
OFFICE OF SCIENCE POLICY
OFFICE OF BIOTECHNOLOGY ACTIVITIES

The National Institutes of Health (NIH) in Bethesda, Maryland, the world's largest medical research facility, is seeking applications from exceptional candidates for the position of Medical Officer, Recombinant DNA Advisory Committee (RAC), located in the Office of Biotechnology Activities (OBA), Office of Science Policy, Office of the Director. The incumbent of this position provides expert clinical advice and assistance to the NIH Recombinant DNA Advisory Committee (RAC), with emphasis on its role in examining clinical trials that involve the transfer of recombinant DNA to humans. Responsibilities include analyzing the need for policy development in recombinant DNA and gene transfer clinical research activities, conducting in-depth analyses of a wide range of recommendations developed by major advisory committees and governmental oversight bodies, and identifying human gene transfer experiments deserving of public discussion by the full RAC.

Candidates are required to have Doctor of Medicine or Doctor of Osteopathy from a school in the United States or Canada approved by a recognized accrediting body. The salary range is $107,521 - $139,774 and a full package of benefits is included. A detailed vacancy announcement with the mandatory qualifications and application procedures can be obtained on **USAJOBS** at **www.usajobs.nih.gov** (**announcement number OD-06-149137**) and the NIH Web Site at **http://www.jobs.nih.gov**. Questions on the application procedures may be addressed to **Laurie Steinman on 301-594-5335**. Applications must be received by midnight eastern standard time on **November 30, 2006**.

WWW.NIH.GOV

CANCER DIAGNOSIS PROGRAM
PROGRAM DIRECTOR
NATIONAL INSTITUTES OF HEALTH
NATIONAL CANCER INSTITUTE

The Cancer Diagnosis Program (CDP) is an extramural program within the Division of Cancer Treatment and Diagnosis of the National Cancer Institute (NCI) responsible for facilitating the translation of new knowledge in cancer biology and technologies into clinically useful diagnostic and predictive tests. CDP initiated the Program for the Assessment of Clinical Cancer Tests (PACCT) in 2000 to ensure that the next generation of biomarkers and laboratory tests improve the management of cancer patients. CDP works closely with other NCI units and with other government agencies that focus on related aspects of the diagnosis challenge. These include the Cancer Therapy Evaluation Program (CTEP), responsible for the NCI's clinical trials program; the Cancer Imaging Program, responsible for improvements in the non-invasive imaging of tumor physiology and biochemistry; staff from various programs involved in the development of state-of-the art informatics systems, and statistical and mathematical techniques adequate for the analysis of massive datasets; other components of the NCI; the National Institute of Standards and Technology; and such regulatory agencies as the FDA. Since the movement of new diagnostic and predictive tests into clinical practice also depends on interactions with the international oncology community, CDP also fosters collaborations with foreign oncology groups.

CDP is seeking an M.D., Ph.D. or D.O. to serve as a Program Director in the Diagnostics Evaluation Branch (DEB) to participate in a dynamic extramural research program of international scope. Experience with clinical trials and an interest in diagnosis and/or predicting the response to treatment, particularly as it relates to evaluation of biomarkers and in vitro diagnostic tools is necessary. The Program seeks an individual with experience in the translation of new knowledge and technology to clinical practice. A knowledge of systems biology and bioinformatics especially as it relates to identification of biomarkers or groups of biomarkers is helpful. Also helpful is experience that involves understanding the clinical decisions that can be informed by the use of markers and molecular technologies. The candidate will work with the Chief of the Diagnostics Evaluation Branch of the CDP and staff in the development of new initiatives for both the academic and business research communities. Significant effort will be devoted to projects initiated as part of PACCT.

Base salary for this position ranges from $91,407 to $118,828 per annum. MD and DO candidates are eligible for an additional allowance beginning at $13,000 per annum, depending on qualifications. Benefits include health and life insurance options, retirement, paid holidays and vacation leave.

To apply for this position, please visit: **http://jobsearch.usajobs.opm.gov/a9nih.asp** and keyword search for Vacancy Announcements **(VA), NCI-06-142673 (Ph.D.) or NCI-06-142674-DH (MD or DO)** for the mandatory application requirements. You must apply by the closing date of **October 30, 2006**. For questions about applying to the VA, please contact **Mary Lou Weathers, on (301) 402-5059 or weatherm@mail.nih.gov**.

For more information about the position, please contact **J. Milburn Jessup, MD at jessupj@mail.nih.gov or (301) 435-9010**.

Cell Cycle Checkpoint Signaling Mechanisms and ATM Function
Richard S. Paules, Ph.D.
Environmental Stress and Cancer Group
(http://dir.niehs.nih.gov/dirlt/paules.htm)

A postdoctoral position is available to investigate signal transduction mechanisms regulating cell cycle checkpoints, with particular interest in the role of the ataxia telangiectasia mutated (ATM) gene product and ATM-interacting proteins. Studies will focus on responses to DNA damage and regulation of cell cycle progression following damage. Current research interests in our group include investigations of the role of ATM in mammary epithelial cell cycle regulation, the interplay of signaling from ATM and ATM-related kinases, as well as ATM signaling in cells from individuals with heritable cancer susceptibility syndromes. Opportunities exist for the incorporation of genomic analyses into studies. Candidates should have a Ph.D. or equivalent degree in molecular biology, cell biology, biochemistry or related field. While candidates with five or fewer years of relevant postdoctoral research experience are eligible, recent graduates are encouraged to apply.

TO APPLY: Submit a cover letter, curriculum vitae, bibliography and the names of three references to:

Dr. Richard S. Paules, National Institute of Environmental Health Sciences, PO Box 12233, (Mail Drop D2-03), Research Triangle Park, NC 27709, paules@niehs.nih.gov, FAX (919) 316-4771.

Postdoctoral, Research and Clinical Fellowships at the National Institutes of Health

www.training.nih.gov/pdopenings

www.training.nih.gov/clinopenings

Train at the bench, the bedside, or both

Office of Intramural Training and Education
Bethesda, Maryland 20892-0240
800.445.8283

Positions @ NIH
THE NATIONAL INSTITUTES OF HEALTH

Department of Health & Human Services (DHHS)
National Institutes of Health (NIH)
National Institute of Dental and Craniofacial Research (NIDCR)

The National Institute of Dental and Craniofacial Research (NIDCR), National Institutes of Health (NIH), Department of Health & Human Services (DHHS) is seeking applicants for a Biologist/Microbiologist/Health Scientist Administrator position in the Center for Integrative Biology and Infectious Diseases (CIBID). The position advertised is for the Director of the Microbiology Program. This program supports extramural basic and translational research on the role of oral microbes in health and disease. To this end, four broad scientific areas provide the basis for rapid development of knowledge of the etiology, pathogenesis, diagnosis, treatment and prevention of oral infectious diseases. These interrelated areas are: (i) Biofilms and Microbial Ecology; (ii) Microbial genomics; (iii) Microbial Virulence and Disease Pathogenesis; and (iv) Prevention and Treatment.

The incumbent will direct, administer and evaluate a portfolio of extramural grants, contracts and cooperative agreements and will stimulate interest in and provide advice to the extramural community regarding the respective research portfolio. In addition, the incumbent will participate in funding decisions, policy development, as well as implementation and coordination with other programs both within and outside of the NIDCR.

The salary range for this position is $77,353 to $118,828 per annum, commensurate with qualifications and professional experience. A full benefits package is available, which includes retirement, Thrift Savings Plan participation, health, life and long-term care insurance.

For qualifications required, evaluation criteria, and application instructions, view the vacancy announcements at: **http://jobsearch.usajobs.opm.gov/a9nih.asp**. Refer to announcement **# NIDCR-06-141634DE** or **NIDCR-06-147841MP**. Applications will be accepted until **October 27, 2006**. Please contact **Michelle Lipinski at 301-594-2286** or **lipinskim@od.nih.gov** if you have questions.

National Human Genome Research Institute

Investigator Recruitment in Cancer Genetics
National Human Genome Research Institute

The Cancer Genetics Branch (CGB) of the National Human Genome Research Institute (NHGRI) is seeking to recruit an outstanding tenure-track investigator to pursue innovative, independent research in cancer genetics. General areas of interest include, but are not limited to:

- Cancer Gene Therapy
- Comparative Cancer Genomics
- Genetic Epidemiology
- Molecular Profiling of Tumors
- Functional Genomics of Cancer
- Genome Instability in Cancer
- Markers for Early Detection
- Genetics of Tumor Progression

The successful candidate will be able to take advantage of interactions with a highly collegial group of scientists within NHGRI and the NIH campus as a whole. In addition, they will have access to NHGRI's outstanding core laboratories.

Candidates must have a Ph.D., M.D., or equivalent degree, as well as comprehensive, advanced training and a record of accomplishment in one of the targeted areas. This position includes generous start-up funds, an ongoing commitment of research space, laboratory resources, and positions for personnel and trainees.

Interested applicants should submit a curriculum vitae, a three-page description of their proposed research, and three letters of recommendation through our online application system at **http://research.nhgri.nih.gov/apply**.

The closing date is **November 17, 2006**.

For more information on CGB and NHGRI's Intramural Program, please see **http://www.genome.gov/Research**. Specific questions regarding the recruitment may be directed to **Dr. William Pavan (Search Chair) at bpavan@mail.nih.gov or by fax at (301-402-2036)**. Questions may also be directed to **Dr. Elaine Ostrander, Chief of the Cancer Genetics Branch, at eostrand@mail.nih.gov or by FAX (301-480-0472)**.

WWW.NIH.GOV

DIRECTOR, GRADUATE PARTNERSHIPS PROGRAM
Office of Intramural Training and Education
National Institutes of Health

The Intramural Research Program of the National Institutes of Health in Bethesda, Maryland is seeking an outstanding individual to serve as Director of the Graduate Partnerships Program (GPP), Office of Intramural Training and Education (OITE). The Director, Graduate Partnerships Program, oversees the organization, implementation, and monitoring of Ph.D.-granting graduate programs associated with the NIH Intramural Research Program and assures that the partnerships are of the highest quality in training, curriculum and student outcomes. Specifically, the GPP is responsible for developing and monitoring policies and procedures and for coordinating institutional support to ensure that graduate student training programs meet the specific criteria of the universities involved in Ph.D.-granting graduate partnerships with the NIH. The incumbent also has a major responsibility for the design and implementation of strategies to facilitate the recruitment of students into the various NIH graduate partnerships. The Director chairs the GPP Executive Committee and oversees an office of ~7 staff. A Ph.D. or equivalent degree plus scientific knowledge and demonstrated expertise in biomedical science as well as experience in running a research group and working with trainees, including graduate and undergraduate students and postdoctoral fellows, is required. A scientific background is also essential for working with NIH and university faculty mentors.

Further details about GPP and specific programs can be found at www.training.nih.gov. The salary range is $107,521 - $139,774 and a full package of benefits is included. A detailed vacancy announcement with the mandatory qualifications and application procedures can be obtained on **USAJOBS at www.usajobs.gov (announcement number OD-06-14059) and NIH Web Site at http://www.jobs.nih.gov**. Questions on application procedures may be addressed to **Laurie Steinman on (301) 594-5335**. Applications must be received by midnight eastern standard time on **December 28, 2006**.

Department of Health and Human Services
National Institutes of Health
Director, National Center for Research Resources and
Associate Director for Clinical Research (Extramural)

The Office of the Director, National Institutes of Health (NIH) in Bethesda, Maryland, is seeking applications from exceptional candidates for the position of Director, National Center for Research Resources (NCRR). The Director, NCRR, will also serve as the NIH Associate Director for Clinical Research (Extramural). NCRR, with a staff of approximately 100 employees and a $1 billion budget, is the focal point at NIH for biomedical, clinical and translational research resources. The incumbent serves as a principal advisor to the Director, NIH; participates in discussions relative to the development of major policy decisions affecting biomedical, clinical and translational research resources; provides advice and consultation to NIH components, advisory councils and grantee organizations and institutions; and assures that effective administrative procedures are established so that program operations and obligations of government funds and other resources are rendered consistent with statutory and regulatory requirements and within limitations imposed by the Department of Health and Human Services (DHHS) and Executive Branch policies. As Associate Director for Clinical Research (Extramural), the incumbent is expected to provide leadership for clinical research activities across the NIH. This leadership will involve the coordination of clinical research activities to enhance the integration of basic and clinical research. The Associate Director for Clinical Research will work closely with the other Institute and Center Directors to enhance the efficiency and effectiveness of clinical research supported by the NIH. Applicants must possess a Ph.D., M.D., or a comparable doctorate degree in the health sciences field plus senior level scientific experience and knowledge of biomedical, clinical and/or translational research programs in one or more health science areas. Salary is commensurate with experience and a full package of benefits (including retirement, health, life, long term care insurance, Thrift Savings Plan participation, etc.) is available. A detailed vacancy announcement, along with mandatory qualifications and application procedures, can be obtained via the NIH Home Page at: **http://www.jobs.nih.gov** under the Senior Job Openings section. Dr. Stephen Katz, Director, National Institute of Arthritis and Musculoskeletal and Skin Diseases, and Dr. David Schwartz, Director, National Institute of Environmental Health Sciences, will be serving as co-chairs of the search committee. Questions on application procedures may be addressed to **Ms. Regina Reiter at ReiterR@od.nih.gov or discussed with Ms. Reiter by calling 301-402-1130**. Applications **must** be received by **November 27, 2006**.

THE UNIVERSITY OF ALABAMA AT BIRMINGHAM

Chair, Department of Biology
School of Natural Sciences and Mathematics

The UAB School of Natural Sciences and Mathematics (NS&M) invites applications for the position of Chair of the Department of Biology. The department presently consists of 17 full-time faculty members with over 900 undergraduate majors and nearly 50 MS and PhD students. Departmental strengths include a highly dedicated and collegial faculty with research interests primarily in the ecology, comparative physiology, and molecular biology of aquatic organisms but also in aspects of cancer biology, immunology, and microbiology, a unique 5th year MS program in biology, and a strong undergraduate honors research program.

The Chair is responsible for the overall departmental program administration and reports to the Dean of the School. The successful candidate will be expected to provide vision and leadership for the department; administer departmental budgets, policies, and facilities; recruit and retain outstanding faculty; further the recruitment of high quality students; contribute directly to scholarly activities of the department; and promote the department both inside and outside the university. The university especially values collaboration among its academic units within and outside of the School that enhances the effectiveness of its academic and research programs. A PhD in Biology or related field is required. Candidates must possess a distinguished record of scholarship, a demonstrated commitment to excellence in teaching, outstanding communication and interpersonal skills, and an established record of university and professional service such as to qualify for a tenured Professor position.

UAB with its world-renowned medical center is the major teaching-research university in the state of Alabama and the only doctoral-granting university in the State to be listed in the top Carnegie category: RU/VH. UAB continually ranks in the top twenty U.S. academic institutions with respect to National Institutes of Health (NIH) funding while NS&M's extramural funding has grown about 15% annually over the past several years. With a total student enrollment of 16,500 and full-time faculty of over 1800, UAB is rapidly becoming one of the state's largest public, comprehensive universities.

Applicants should submit a letter of interest summarizing their qualifications, curriculum vitae, statement of vision, other supporting documentation, and contact information for at least five references. Screening will begin in December 2006 and continue until a suitable candidate has been selected. Please send applications to: **Department of Biology Search Committee, School of Natural Sciences and Mathematics, 1530 3rd Avenue South, CH 464, University of Alabama at Birmingham, Birmingham, AL 35294-1170**.

Women and minorities are strongly encouraged to apply.
UAB is an Affirmative Action, Equal-Opportunity Employer.

Research Faculty Scientists
Institute for Transfusion Medicine (ITxM)

The Institute for Transfusion Medicine (ITxM) is now accepting applications for Research Faculty Scientists (all levels) in its new Center For Research (CFR) focusing on basic physiology and pathology of blood and transfusion-related topics. Joint academic appointments will be provided by the Department of Pathology, University of Pittsburgh. ITxM is the fourth largest blood center in the US and manages the largest transfusion service in the country. The ITxM CFR seeks to complement its existing strong clinical program and extensive clinical and translational research portfolio with an independent laboratory based investigative research program. MD and/or PhD trained scientists with an interest in molecular immunohematology and/or cellular therapy are particularly encouraged to apply. The successful candidate should have a track record of extramural funding or early career progress indicating a commitment to developing an externally funded research program. ITxM will provide very competitive start up packages and newly renovated laboratory facilities.

Please send a research statement, CVs, and list of potential references to: **Deborah Small, Institute for Transfusion Medicine, 3636 Blvd of the Allies, Pittsburgh, PA 15213**. Inquiries can be addressed to **Professor Darrell J Triulzi (dtriulzi@itxm.org)** or **Professor Alan Wells (wellsa@upmc.edu)**.

ITxM is an Equal Opportunity Employer.

University of California, Berkeley
Berkeley Nanosciences and Nanoengineering Institute (BNNI)
Assistant Professor Position in Nanoscale Science and Engineering and Energy

The University of California, Berkeley solicits applications for a tenure track position of Assistant Professor beginning in the Fall of 2007. Candidates are sought in the fields at the intersection of nanoscale science and engineering with implications of energy technology. Many of the fundamental length scales involved in energy conversion, transmission, and storage occur at the nanoscales. Therefore, nanoscale science and engineering provide the opportunity to discover and develop new processes and systems to cost-effectively convert, store, and transmit energy that significantly reduces the atmospheric burden of greenhouse gases. Topics of interest include but are not limited to catalysis, surface adsorption, fuel synthesis and processing including biofuels, bioprocessing, thermoelectrics, fuel cells, batteries, photovoltaic cells, superconducting power transmission, clean coal conversion, nuclear power, etc.

This faculty search will be conducted under the auspices of the Berkeley Nanosciences and Nanoengineering Institute (BNNI), with participation from the Departments of Physics, Chemical Engineering, Chemistry, Electrical Engineering and Computer Science, Materials Science and Engineering, and Mechanical Engineering. The successful candidate will have the potential to interact with scientists and engineers across a wide spectrum of disciplines, and to help develop the new interdisciplinary initiative in nanoscale science and engineering. Applicants should send a complete curriculum vitae, a selection of publication reprints (five or less), and a brief statement of future research plans and teaching interests. Candidates should also provide the names of at least three references to the address below. Applicants should request that their references forward letters to the same address. Such letters will not be requested directly by the department or the committee. UC Berkeley's Statement of Confidentiality can be found at **http://apo.chance.berkeley.edu/evalltr.html**.

Applications should be sent to: **Chair, Faculty Recruitment Committee; Berkeley Nanosciences and Nanoengineering Institute, c/o Department of Materials Science and Engineering, University of California, MC1760; Berkeley, CA 94720**. The deadline for receipt of applications, including references, is **December 1, 2006**.

The University of California is an Equal Opportunity/ Affirmative Action Employer.

DAVID GEFFEN SCHOOL OF MEDICINE UCLA

PHYSIOLOGY UCLA
Assistant or Associate Professor

The **Department of Physiology** at the **David Geffen School of Medicine at UCLA** invites applications for a tenure track faculty position, preferably at the level of Assistant or Associate Professor.

We are especially interested in candidates using molecular physiological approaches such as functional genomics, proteomics, molecular imaging, or systems biology. Areas of departmental strength include **molecular biophysics** and **cardiovascular research**, and candidates in these disciplines are encouraged to apply. However, we will consider applicants in all areas of modern physiology. Areas in which we might hope to expand include renal and respiratory physiology. Candidates are expected to have a strong background in cellular and molecular biology and a demonstrated interest in addressing fundamental physiological problems.

The successful candidate will be expected to develop an independent research program and participate in the teaching mission of the Department.

Interested applicants should email their curriculum vitae, a letter with a statement of research interests and career goals, and the names of three references to **Dr. Ernest Wright** at **PhysiologySearch@mednet.ucla.edu**. Applicants should also arrange for three letters of reference to be sent to Dr. Wright at the same email address.

UCLA is an Affirmative Action/Equal Opportunity Employer.
Women and minorities are encouraged to apply.

The Gerstner Sloan-Kettering Graduate School of Biomedical Sciences offers the next generation of basic scientists a program to study the biological sciences through the lens of cancer — while giving students the tools they will need to put them in the vanguard of research that can be applied in any area of human disease.

PhD Program

An Internationally Recognized Research Faculty in:

- Cancer genetics
- Genomic integrity
- Cell signaling and regulation
- Structural biology
- Immunology
- Chemical biology
- Developmental biology
- Computational biology
- Experimental therapeutics
- Experimental pathology
- Imaging and radiation sciences
- Oncology
- Genomics
- Animal models of disease

An Innovative, Integrated Curriculum Provides a Fundamental Understanding of:

- The nature of genes and gene expression
- Cellular organization
- Tissue and organ formation
- Cell-cell interactions
- Cellular response to the environment
- Enzyme activity

Student Support and Services:

Students receive a fellowship package that includes a stipend, tuition, textbook allowance, and health insurance. Students also have access to affordable housing within easy reach of the school.

Please visit our Web site to learn how to apply, for application deadlines, and for more information about our PhD program.

www.sloankettering.edu

gradstudies@sloankettering.edu | 646.888.6639

Gerstner Sloan-Kettering
Graduate School of Biomedical Sciences

New York City

PRESIDENT, MEDSTAR RESEARCH INSTITUTE
MedStar Health
Washington, DC

MedStar Health is conducting a national search to select the next President to lead the MedStar Research Institute (MRI). The Research Institute is the research center that supports the academic platform of MedStar Health with a budget of $50 million. The mission of the Research Institute is to provide the scientific, administrative, and regulatory support for the research programs and initiatives, which complement and advance key clinical services and teaching programs among the 7 hospitals in the MedStar hospital network. The Institute works in close collaboration with MedStar's major academic partner, Georgetown University.

MedStar Research Institute ranks in the top 10% of research institutes nationally for total NIH funding. MedStar Health's 7 hospitals, which include Georgetown University Hospital and the Washington Hospital Center, in addition to community teaching hospitals and a nationally known rehabilitation hospital, are all located within the Baltimore/Washington corridor, offering 36 Centers of Excellence and many nationally top-ranked programs providing high quality care to more than 130,000 inpatients and 905,000 outpatients annually.

This position represents an excellent opportunity for a senior-level researcher or physician-scientist leader to build and enhance MedStar Health's translational research programs. The President will be the major research liaison for MedStar with Georgetown University. The President will: lead and direct MedStar Health's research agenda both within the Institute and at MedStar hospitals, work with senior MedStar executives and physicians to foster collaborative, interdisciplinary research efforts, reinforce MRI's role as a research service organization, cultivate increased funding, and develop relationships with peers at external scientific, healthcare, and regulatory entities.

Qualified candidates for this position will possess an MD and/or PhD as well as the academic credentials appropriate for appointment at the rank of professor. Successful candidates will have a strong research background and history of peer-reviewed funding and publications. Candidates should be highly skilled at driving interdisciplinary collaborative efforts across organizations, as well as possess the financial and operational acumen required to effectively manage and lead a research institute.

Korn/Ferry International is assisting the MedStar Research Institute in its search. Please forward nominations and self-nominations of appropriate candidates to: **Renée K. Akin, Associate (renee.akin@kornferry.com)** or **David M. Shabot, Senior Client Partner (david.shabot@kornferry.com); Korn/Ferry International, 1835 Market St., Ste. 2000, Philadelphia, PA 19103; 215-656-5360 (phone); 215-496-0195 (fax).**

MedStar Health and Korn/Ferry International are Equal Opportunity/Affirmative Action Employers.

INDIANA UNIVERSITY SCHOOL OF MEDICINE

DIRECTOR - CENTER FOR AIDS RESEARCH

Indiana University is recruiting for the position of Director for the IU Center for AIDS Research. The position will include a faculty appointment at the appropriate rank within the Department of Medicine and/or in the Department of Microbiology & Immunology. We seek a candidate who has a well-funded HIV virology research program, who will take a lead role in expanding the Center's basic and translational research. The candidate's research could include pathogenesis, immunology, vaccine development or drug resistance. Indiana University has a core of scientists whose focus includes HIV immunology, pharmacokinetics, neuropathogenesis, chemokine receptor usage, nephropathy and metabolic dysregulation, an active AIDS Clinical Trials Unit and an expanding HIV program in western Kenya. Appropriate administrative and research space, funds to recruit 4-6 additional faculty, and institutional support for the Center are available. Candidates must have a Ph.D. or M.D. degree (or equivalent). For consideration, send a CV, letter of interest, statement of research interest, and the names of 3 references electronically to **virsch@iupui.edu** or by mail to: **Kenneth H. Fife, MD, Ph.D., IU Center for AIDS Research, 545 Barnhill Drive, Room 435, Indianapolis, IN 46202-5124**.

Electronic submissions are encouraged.

Indiana University is an EEO/AA employer, M/F/D

Disease Ecologist

The Department of Biology at Emory University is seeking to recruit an ecologist who studies the fundamental ecological processes affecting the dynamics, spread and emergence of infectious diseases. The department will consider applicants holding a Ph.D. or equivalent degree from a wide range of specializations including, but not limited to: (i) theoretical and/or experimental work on the population dynamics of infectious diseases and/or the immune response within a host; (ii) molecular ecology and epidemiology of infectious disease; and (iii) experimental and field studies of pathogen-host interactions. The position is for a tenure-track assistant professor, although an appointment at a higher rank will be considered in exceptional circumstances. Applicants must provide evidence that they will develop a strong, independently funded research program. A commitment to undergraduate teaching is expected and the appointees will also participate in appropriate Ph.D. granting programs of the interdepartmental Graduate Division of Biological and Biomedical Sciences.

Applicants should submit a curriculum vitae and a statement detailing their current and future research plans, and arrange for submission of three letters of recommendation. Please address applications to: **Dr. Leslie A. Real, Disease Ecology Search, Department of Biology, Emory University, 1510 Clifton Road, Atlanta, GA 30322; Tel: (404) 727-4234; Fax: (404) 727-2880; E-mail: biol_srch@emory.edu**. Review of completed applications will begin **November 15, 2006**. Information on the Biology Department can be found at **http://www.biology.emory.edu** and a description of the graduate programs in the Graduate Division of Biological and Biomedical Sciences is provided at **http://www.biomed.emory.edu/**.

Emory is an Equal Opportunity/Affirmative Action Employer.

MRC | Laboratory of Molecular Biology

MRC Laboratory of Molecular Biology

Group Leader Position in Molecular Biology

The Protein and Nucleic Acid Chemistry Division of the MRC Laboratory of Molecular Biology would like to attract applications for a group leader position from molecular biologists who exploit genetic approaches (using mouse or cell-line models) to dissect fundamental processes in vertebrate biology. Applications from candidates working with the immune or haematopoietic systems will be especially favoured.

Appointment of a junior-group leader would be at programme-leader track level although appointment at a more senior level to a tenured, programme leader position will also be considered.

Current interests of groups in the Division can be found at (http://www2.mrc-lmb.cam.ac.uk/PNAC). These activities span from X-ray crystallography, mammalian molecular cell biology, immunobiology and transgenic mouse models through to biotechnology. Further information about this position is available at http://www2.mrc-lmb.cam.ac.uk/groupleaderinfo.html

Applications should include a covering letter and full CV, and an outline of current and future research interests, along with the names and addresses of three professional referees who have agreed to be contacted prior to interview. Weight will be attached to the originality and nature of the project proposal, which should aim to tackle a fundamental and/or important problem. Enquiries are welcome at any time but, for this recruitment, please reply by 15th November 2006.

Quoting job reference PNAC/906/6, please e-mail your application to: recruit@mrc-centre.cam.ac.uk or post to: Recruitment Office, Personnel Department, MRC Centre, Hills Road, Cambridge CB2 2QH, UK.

Closing date: 15th November 2006

This is a No Smoking site.
For further information about MRC, visit www.mrc.ac.uk
The Medical Research Council is an Equal Opportunities Employer.
'Leading Science for Better Health'

Cincinnati Children's Hospital Medical Center

Developmental Mechanisms, Organogenesis, and Stem Cell Research

Faculty positions at Assistant, Associate, and Full Professor levels

Cincinnati Children's Hospital Medical Center, one of the world's top ranking pediatric research centers, is making a major investment in basic and translational research in developmental sciences. The Division of Developmental Biology, under the Directorship of Chris Wylie, will recruit up to twenty additional tenure and tenure-track faculty, to form interdisciplinary groups of basic scientists and clinicians who will study the fundamental mechanisms of development and their application to congenital and acquired childhood disease.

Faculty will occupy outstanding research space in a new 400,000 sq.ft. building, which will be ready in Fall 2007.

Faculty at CCHMC have access to high-quality graduate programs, an MD/PhD program, and training programs for postdoctoral fellows, clinical residents and fellows.

Applications are invited for the first of these positions in the following areas of research:

Fundamental Mechanisms of Development: Candidates working on the molecular and cellular mechanisms of development, using any model animal system, are encouraged to apply.

Organogenesis: Candidates working on the development of the following organ systems are particularly encouraged to apply. Several appointments will be made in each of these areas. Appointment at a senior level could include leadership opportunities in further recruitments.

- **Skeletal System**
- **Kidney and Urinary Tract**
- **Skin**
- **Vascular System**

Stem Cells: Candidates working on any aspect of stem cell research, using any animal model, are encouraged to apply. Up to eight appointments will be made in this area, including a leadership position.

Protein Sciences: We welcome applicants in the general areas of biochemistry, chemical biology, protein engineering/evolution/design, and structural biology who share an interest in studying the molecular basis, diagnosis and treatment of pediatric disorders.

Interested candidates should hold a PhD, MD, or MD/PhD. Please send curriculum vitae, two page research statement, and contact information for three people who will provide letters of recommendation to: **DOSpositions@cchmc.org**

Candidates should indicate in their cover letters the position(s) in which they are particularly interested.

The Cincinnati Children's Hospital Medical Center, and the University of Cincinnati are Affirmative Action/Equal Opportunity Employers. Qualified women and minority candidates are especially encouraged to apply.

Please visit our web site at: **www.cincinnatichildrens.org/research/div/dev-biology**

Senior Lecturer/Associate Professor, Developmental Biology

Anatomy and Cell Biology
Faculty of Medicine, Nursing and Health Sciences

Monash University, Melbourne, Australia

We are looking for an outstanding person with a record of excellence in developmental biology research and teaching. The appointee will provide leadership in the development and delivery of a new BSc major in developmental biology. This major covers topics such as normal development and birth defects, mechanisms of development, and stem cells and regeneration. The appointee is expected to have competitive research funding and a recognised research reputation.

Salary range: $A78,309 – $A90,294/$A94,291 – $A103,877 pa Level C/Level D plus generous superannuation

Contact: Professor John Bertram, tel. +61 3 9905 2751 or email john.bertram@med.monash.edu.au

Applications: By mail addressed to Ms Chris Kelly, Department of Anatomy and Cell Biology, Monash University, Building 13C, Clayton Victoria 3800, Australia or email chris.kelly@med.monash.edu.au by 27/10/2006.

Location: Clayton campus **Ref No:** A067148

Prospective applicants are required to refer to **www.monash.edu.au/opportunities** for position description/selection criteria and information on how to apply.

Monash respects the privacy of your personal information. For more details visit www.privacy.monash.edu.au

An Equal Opportunity Employer EOWA Employer of Choice for Women

MONASH University

UNC
N.C. CANCER HOSPITAL
LINEBERGER COMPREHENSIVE CANCER CENTER

Scientist Position

The UNC Lineberger Comprehensive Cancer Center is searching for promising or established scientists for tenure-track positions in the broad areas of cancer biology including tumor virology. Candidates should have a Ph.D. or M.D. with a strong record of recent accomplishments as a postdoctoral fellow or sustained productivity as an established faculty member. The University of North Carolina at Chapel Hill department and rank will be determined by the applicant's qualifications. The search will be coordinated by Albert Baldwin, Nancy Raab-Traub and Yue Xiong.

Areas of interest include but are not limited to: tumor virology, bioinformatics and computational biology, stroma/tumor cell interaction, developmental and stem cell biology, angiogenesis, metastasis, growth control, nuclear hormone receptors, molecular therapeutics, and genetics of cancer predisposition. Applicants should send a curriculum vitae, a description of research plans, and three letters of reference to: **Melissa Stroud Mack, UNC Lineberger Comprehensive Cancer, Center CB# 7295, University of North Carolina at Chapel Hill, Chapel Hill, NC 27599-7295.** The committee will begin reviewing applications **December 15, 2006**.

The University of North Carolina at Chapel Hill is an Equal Opportunity/ADA Employer. Women and minorities are encouraged to apply.

UNIVERSITY OF MICHIGAN
CENTER FOR
stem cell biology
lifesciences institute

The Life Sciences Institute and the University of Michigan Medical School invite applications for tenure track **ASSISTANT PROFESSOR** positions. We are seeking outstanding scholars, with Ph.D., M.D. or equivalent degrees and relevant postdoctoral experience, who show exceptional potential to develop an independent research program that will address fundamental issues in any aspect of stem cell biology. Applicants who have already established successful independent research programs will be considered for tenured **ASSOCIATE PROFESSOR** or **PROFESSOR** positions.

Applicants should send a curriculum vitae, copies of up to three reprints, a one- to two-page summary of research plans, and arrange to have three letters of reference sent directly by November 1, 2006 to: **Stem Cell Search Committee, c/o Rebecca Fritts, Life Sciences Institute, University of Michigan, 210 Washtenaw Avenue, Ann Arbor, Michigan, 48109-2216**.

The University of Michigan is an Affirmative Action/Equal Opportunity Employer.

The newly founded Interdisciplinary Center for Molecular Materials (ICMM) offers the position of

W2-Professor for Computational Chemistry

at the earliest possible date. The position is located at the Institute for Physical and Theoretical Chemistry/Center for Computational Chemistry in the Faculty of Natural Sciences II (Biology, Chemistry, Pharmacy).

Candidates will be expected both to teach and carry out research in computational chemistry at a high level. Research interests may be in the general area of computer-aided investigations of large molecular or supramolecular systems, particularly those related to materials science. Possible research areas include, but are not limited to, the application and development of molecularmodeling methods, QM/MM-techniques or those based on Born-Oppenheimer ar Car-Parinello molecular dynamics.

Qualifications include university undergraduate and doctoral degrees, good teaching skills, and a habilitation or equivalent other qualification, which may have been gained outside the University or within a "Juniorprofessur".

At the time of appointment the candidate must not be older than 52 years of age. The Ministry for Science, Research and Art may allow an exception in special cases, which has to be approved by the Ministry of Finance.

The University of Erlangen-Nuremberg actively encourages applications from female candidates in an effort to increase female representation in research and teaching.

Applications from the severly disabled having the same suitability for appointment as other candidates will be given priority.

Application documents (curriculum vitae, photograph, list of publications and teaching activities, certified copies of degree certificates but no publications) must be sent by the latest **November 6, 2006** to: Dekan der Naturwissenschaftlichen Fakultät II (Biology, Chemie und Pharmazie) der Universität Erlangen-Nürnberg, Universitätsstr. 40, 91054 Erlangen, Germany.

Friedrich-Alexander-University Erlangen-Nuremberg

www.uni-erlangen.de

7 T MR

Professor of MR Physics (m/f)

Radiology

At the Leiden University Medical Center (LUMC), we are continually seeking to improve the quality of healthcare. The LUMC aims for excellence in patient care, research, teaching, training and continuing education. Its Department of Radiology is seeking a Professor of MR Physics to lead its 7.0 Tesla MR Physics group.

■ The Department of Radiology has 1.5 and 3.0 Tesla MRI scanners and, in the coming year, a 7.0 Tesla scanner will be installed. The ultra high field MR research to be carried out will be on molecular imaging and functional imaging, and an important applicatory focus will be on neurodegenerative disorders.

■ As **Professor of MR Physics**, you will be responsible for all research carried out on the 7.0 Tesla MR scanner. The position presents an opportunity to develop a new line of MR physics research within the LUMC with funding available to recruit three additional MR physicists (tenured positions). The position demands ample experience with high-field human MR physics, and experience with the Philips MR systems and the Philips pulse programming environment is an advantage. In addition, you should have proven expertise in grant applications and in the supervision of PhD students and postdocs. The appointment is full time and tenured.

■ A detailed description of this position (together with full terms of employment) can be found on **www.lumc.nl** (select **'Job opportunities'**). Informal enquiries can be obtained from Professor M.A. van Buchem, MD, Department of Radiology, +31 (0)71 526 43 76, or M.J.P. van Osch, PhD, Department of Radiology, +31 (0)71 526 36 78.

■ To apply, please send your letter of application and CV to LUMC, Attn. Professor M.A. van Buchem, MD, Human Resources Division 2, LUMC K5-S, PO Box 9600, 2300 RC Leiden, the Netherlands. The closing date for applications is 1 December 2006. Please quote ref. no. B.06.PV.12/SC40 both on the envelope and on the letter. Alternatively, you can e-mail your application and CV to div2peno@lumc.nl.

LUMC
PO Box 9600
2300 RC Leiden
Tel. +31 (0)71 526 91 11

LUMC

WWW.LUMC.NL

New York University

FACULTY POSITIONS

Department of Biology

CENTER FOR COMPARATIVE FUNCTIONAL GENOMICS

NEW YORK UNIVERSITY
CENTER FOR COMPARATIVE
FUNCTIONAL GENOMICS

As part of a multi-year hiring plan, New York University's Center for Comparative Functional Genomics in the Department of Biology invites applications for multiple faculty positions (rank open) to begin September 1, 2007, or as negotiated, pending budgetary and administrative approval. Candidates using high throughput approaches and computational methods to investigate biological regulatory mechanisms and their evolution at the level of systems and networks are especially encouraged to apply. Candidates will be expected to have or develop active, externally funded research programs and to participate in the department's teaching activities at both the undergraduate and graduate levels. The Center and the Department (http://www.nyu.edu/fas/dept/biology) offer an outstanding and collegial research environment and opportunities for active collaborations with other related divisions within the university including the NYU Courant Institute's Departments of Mathematical and Computer Science and with genomic consortia formed with other New York institutions.

Applications should include cover letter, research statement, curriculum vitae, and three letters of reference. Electronic applications as PDF files should be sent to **biology.recruitment@nyu.edu**. or **Chair of the Search Committee, New York University, Center for Comparative Functional Genomics, Department of Biology, New York University, 1009 Silver Center, 100 Washington Square East, New York, N.Y. 10003**. *Deadline will be* ***November 30, 2006***.

NYU is an Equal Opportunity/Affirmative Action Employer.

Oklahoma State University
Four Tenure-Track Positions

As part of our strategic plan to strengthen research foci in Environmental Stress and Ecology and Evolutionary Biology, the Department of Zoology at Oklahoma State University invites applications for four tenure-track faculty positions; one at open rank in the area of Environmental Stress and three at the assistant professor level in either Environmental Stress or Ecology and Evolutionary Biology. We are seeking a complementary set of scientists with expertise in, but not limited to, areas such as ecotoxicology, invasive species, multiple stressors, ecology of infectious diseases, behavioral ecology, physiological ecology, population genetics, or evolutionary developmental genetics. The ideal candidates will have a Ph.D., post-doctoral research experience, teaching experience, and success in obtaining extramural funding. Each position's responsibilities include establishing a vigorous, extramurally funded research program, successfully mentoring M.S. and Ph.D. students, developing a graduate course in the candidate's area of expertise, and teaching an additional course at the undergraduate level.

The Department of Zoology has a long history of research in conservation biology, integrative ecology, and environmental toxicology, and a variety of partnerships with the departments of Botany, Microbiology and Molecular Genetics, Biochemistry and Molecular Biology, the newly created Department of Natural Resource Ecology and Management, the Oklahoma Cooperative Fish and Wildlife Research Unit, and the College of Veterinary Medicine. More information can be found at **http://zoology.okstate.edu**. Departmental growth coincides with expansion on the University campus as a whole, including construction of an Integrated Science Building scheduled for completion in 2008. Successful candidates can expect competitive salaries and start-up packages, and to be joined by additional faculty, including two senior researchers, to be hired within the next three years.

Candidates should submit (preferably by e-mail) a letter of application, curriculum vitae, statement of research and teaching interests, names and contact information for three references, and up to three sample publications to: **Dr. Joe Bidwell (joe.bidwell@okstate.edu), Interim Head, Department of Zoology, Oklahoma State University, 430 Life Sciences West, Stillwater, OK 74078**. Application review will begin **1 November 2006** and continue until the positions are filled, with employment beginning in August 2007. *Women and minorities are strongly encouraged to apply. Oklahoma State University is an Equal Opportunity/Affirmative Action Employer.*

Sandia National Laboratories

A Department of Energy National Laboratory

Harry S. Truman Research Fellowship
In National Security Science and Engineering

Sandia National Laboratories is one of the country's largest research facilities employing nearly 8,600 people at major facilities in Albuquerque, New Mexico and Livermore, California. Please visit our website at www.sandia.gov.

We are searching for outstanding Ph.D. candidates to apply for the Harry S. Truman Research Fellowship in National Security Science and Engineering. This initial one-year appointment may be extended, at management's discretion, for two additional one-year appointments. The salary is $96,100 per year. This position requires a United States Department of Energy Security Clearance, which requires United States Citizenship.

The Truman Fellowship provides the opportunity for recipients to pursue independent research of their choosing that supports Sandia's national security mission. Candidates are expected to have solved a major scientific or engineering problem in their thesis work or will have provided a new approach or insight to a major problem, as evidenced by a recognized impact in their field.

Candidates must have a Ph.D. within the past 3 years or, will complete all Ph.D. requirements by commencement of appointment, with a broad-based background and extensive knowledge of research in one or more of the following areas: biotechnology; chemical and earth sciences; computing; mathematics and information sciences; electronics and photonics; microsystems and engineering sciences; manufacturing science and technology; materials sciences, pulsed power/directed energy; and robotics and intelligent systems. Candidates must be seeking their first national laboratory appointment, have excellent academic and research qualifications, good communication skills, and enjoy working in a team-oriented, dynamic environment.

For complete instructions, please visit:
http://www.sandia.gov/employment/special-prog/truman.
Please submit the complete package to: Roberta Rivera, Sandia National Laboratories, P.O. Box 5800 MS: 1351, Albuquerque, New Mexico 87185-1351, or email rjriver@sandia.gov, or fax 505-845-9802. Please reference Job Requisition Number: 055490. All materials must be received by December 5, 2006.

U.S. Citizenship Required. Equal Opportunity Employer. M/F/D/V.

LOCKHEED MARTIN

Faculty Position
in Bioinformatics
University of Maryland Baltimore County (UMBC)

The Department of Biological Sciences at UMBC invites applications for an Assistant Professor position in the area of Bioinformatics. A successful applicant will be expected to establish a vigorous externally funded in silico research program focused on one or more significant biological problems in the areas of genome biology or proteomics, including interactomics. An interest in developing tools to visualize complex bioinformatics data sets is also desirable. We will consider appointment of qualified candidates at the Associate or Full Professor level.

Applicants should provide a curriculum vitae, summary of current and proposed future research interests, and statement of teaching interests, and have three letters of reference sent to: **Search Committee, Department of Biological Sciences, University of Maryland Baltimore County, 1000 Hilltop Circle, Baltimore, MD 21250**. Electronic applications should be sent to **biosearch@umbc.edu**. Review of completed applications will begin on **November 15, 2006** and continue until the position is filled.

UMBC is a medium-sized research university in the Baltimore-Washington, D.C. area combining excellence in research with outstanding educational programs. UMBC is a national leader in mentoring minority undergraduates to high achievement in academics and research. The Department is developing in the area of bioinformatics to complement its existing strengths in molecular, cellular, developmental and evolutionary biology and neuroscience. For information about the Department and its graduate programs visit **http://www.umbc.edu/biosci/**.

The University of Maryland Baltimore County is an Affirmative Action/Equal Opportunity Employer. UMBC values gender, ethnic, and racial diversity; women, members of ethnic minority groups and individuals with disabilities are strongly encouraged to apply.

University of Bergen

is a city university. Parts of the campus are in fact situated in the town centre. We have about 17.000 students and nearly 3000 employees. UiB is renowned for its research which holds a high European standard and we have three Centres of Excellence (CoE). The University of Bergen has a strong international profile which entails close co-operation with universities all over the world.

Faculty of Mathematics and Natural Sciences - Department of Earth Science

Professor of Earthquake Seismology, University of Bergen, Norway

The position requires international stature in the field of earthquake seismology. Applicants must have a strong established international record of research in earthquake seismology with an emphasis on theoretical and methodical aspects. Experience in application of seismological methods and data, from regional and global networks to study the structure, composition, deformation and processes in the Earth's interior is considered important.
Furthermore, it is desirable that the research interests of the applicant complement and enhance the activities in the geodynamics research group at the department of Earth Science. The successful candidate must document ability to cooperate with colleagues within and outside of earthquake seismology, as well as ability to direct research teams.

For further details see http://www.uib.no/stilling, or contact Professor Olav Eldholm, Head of Department (olav.eldholm@geo.uib.no; phone +47 55583219).

Application in five copies, sorted into five bundles, is to be submitted to the **Department of Earth Science, The University of Bergen, Allég. 41, NO-5007 Bergen, Norway, by 1 November 2006, marked 06/763/MN.**

CICERO ab

Senior Faculty Position - Director of Research
Department of Ophthalmology and Visual Science
Yale University School of Medicine

The Department of Ophthalmology and Visual Science at Yale School of Medicine invites applications for a full-time faculty position at the level of Professor with tenure. The successful candidate will serve as the Department's Director of Research. He/she will be expected to lead the development of a new multi-investigator program that emphasizes the translational aspects of vision research and builds on Yale's current interdepartmental strengths in the neurobiology and genetics of vision. Areas of particular interest include macular degeneration, vascular biology/angiogenesis, and retinal cell biology.

Candidates must have a Ph.D. and/or M.D.degree, a strong track record of accomplishment at the forefront of vision research, and well-developed leadership and management skills.

Send C.V., statement of interest, copies of relevant publications, and contact information for three references to:

James C. Tsai, M.D.
Professor and Chair
Department of Ophthalmology and Visual Science
Yale University School of Medicine
P O Box 208061
New Haven CT 06520-8061
or
James.Tsai@Yale.edu

The University of Texas at Austin

Endowed Chair Positions

The Institute for Cellular and Molecular Biology

The Institute for Cellular and Molecular Biology invites applications for Endowed Chair positions. Academic appointments at the level of tenured Professor will be held in an appropriate academic unit in the College of Natural Sciences. Candidates should have an outstanding research program that applies molecular biological and/or biochemical approaches to important biological problems. The positions carry exceptional salaries and start-up packages.

Building on a strong existing faculty, the Institute has recruited more than 40 new faculty members in the past eight years. Faculty roster can be viewed at: http://www.icmb.utexas.edu. In addition to it's highly interactive and interdisciplinary research environment, the Institute is the home base for the University-wide Graduate Program in Cell and Molecular Biology and supports the state-of-the-art core facilities for DNA and protein analysis, mass spectrometry, electron and confocal microscopy, DNA microarrays, robotics, and mouse genetic engineering. A recently instituted MD-PhD program with the UT Medical Branch and the forthcoming Dell Pediatrics Research Institute will further enhance the environment for Biomedical Research.

Austin is located in the Texas hill country and is widely recognized as one of America's most beautiful and livable cities.

Please send curriculum vitae, summary of research interests, and names of five references to:

Dr. Alan M. Lambowitz, Director
Institute for Cellular and Molecular Biology
The University of Texas at Austin
1 University Station A4800
Austin TX 78712-0159

Homepage • http://www.icmb.utexas.edu

The University of Texas at Austin is an Equal Opportunity Employer. Qualified women and minorities are encouraged to apply; a background check will be conducted on applicant selected.

Faculty Position in Microbial Genomics University of Illinois at Urbana-Champaign

Applications are invited for a position in microbial genomics in the Departments of Food Science and Human Nutrition, Animal Sciences, or Chemical and Biomolecular Engineering, as part of the campus interdisciplinary initiative in genomic biology, and more specifically, the molecular bioengineering of biomass conversion theme associated with the Institute for Genomic Biology (IGB). The position is at the Assistant or Associate Professor level. The areas of interest are the application of microbial functional genomics to biochemical engineering, cellular engineering of biomolecules and pathways, metabolic engineering, and metabolic flux in industrial microbes. Application of these areas to microbial degradation of plant cell walls for production of biofuels is of special interest. Further information about qualifications and responsibilities is available at **http://www.traill.uiuc.edu/jobsearch/microbial/**. The University of Illinois at Urbana-Champaign is a world-class institution with quality academic units including a center of excellence in biotechnology and a national supercomputing center. Successful candidates will be provided with excellent laboratory facilities, and substantial start-up funds. The position will be available January 16, 2007.

Applicants should submit a curriculum vitae with complete list of publications, a concise summary of research and teaching accomplishments and future plans, and provide three letters of reference. Applications in the form of a single PDF file should be submitted to: **http://www.traill.uiuc.edu/jobsearch/microbial/**. To ensure full consideration, applications must be received by **November 15, 2006**. Questions can be directed to **Dr. Hans P. Blaschek**, Chair of Search Committee, at **217-333-8224** or **blaschek@uiuc.edu**. Additional information about the Institute for Genomic Biology and the Departments or the University can be found at **http://www.igb.uiuc.edu/index.html**, **http://www.ansci.uiuc.edu**, **http://www.scs.uiuc.edu/chem_eng/**, and **http://www.fshn.uiuc.edu**.

The University of Illinois is an Affirmative Action, Equal Opportunity Employer.

TENURE TRACK POSITION
Applied Physics - Req. #05859

The School of Applied and Engineering Physics at Cornell University is seeking applications for a tenure-track, assistant professor position. Consideration of applications for an associate or full professor level position may also be given to exceptionally well qualified individuals. Candidates must be able to demonstrate the ability to develop a highly successful independent research program in an area of applied physics, and to participate effectively in the teaching of the applied physics curriculum at both the undergraduate and graduate level. Research areas of interest in this search include, but are not limited to, optics and photonics, biological physics, nanostructure science and technology, novel instrumentation methods, computational physics, and materials physics. Prospective candidates who wish to pursue interdisciplinary research efforts are strongly encouraged to apply. The successful applicant can expect a very competitive level of support for the start-up of a research program. Considerable institutional resources are available at Cornell that can strengthen this research program and support interdisciplinary and collaborative research ventures. The successful candidate can expect to benefit from association with one or more of Cornell's interdisciplinary research centers, national facilities, and national resources, listed at: **http://www.engineering.cornell.edu/research/research-centers/**

Applications consisting of a resume, a statement of teaching philosophy, a brief (3-page limit) statement of research interests, and the names and addresses of at least three references, should be submitted either on-line, at: **http://fast.aep.cornell.edu/,** or in hard copy to: **Faculty Search Committee, School of Applied and Engineering Physics, 212 Clark Hall, Cornell University, Ithaca, New York 14853-2501.**

Review of applications will begin on December 1, 2006. Interviewing will begin after January 1, 2007, and will continue until the position is filled.

Cornell University

Cornell University is an Equal Opportunity, Affirmative Action educator and employer.

http://chronicle.com/jobs/profiles/2377.htm

One of the oldest institutions of higher education in this country, the University of Delaware today combines tradition and innovation, offering students a rich heritage along with the latest in instructional and research technology. The University of Delaware is a Land Grant, Sea-Grant, Urban-Grant and Space-Grant institution with its main campus in Newark, DE, located halfway between Washington, DC and New York City. Please visit our website at **www.udel.edu.**

Assistant Professor
Molecular Physiology

The Department of Biological Sciences at the University of Delaware invites applications for a tenure-track faculty position at the Assistant Professor level in the area of molecular physiology with particular emphasis on regenerative or developmental biology. A strong focus in molecular and/or genetic techniques is required and research in fish or amphibian models is preferred. The starting date for this position is September 1, 2007 or later.

Requirements for the position include a Ph.D. or equivalent degree and a minimum of two years postdoctoral experience. The person hired will be expected to develop an active research program, pursue extramural funding, and participate in undergraduate and graduate education. The successful candidate will occupy recently renovated lab space with an attached aquarium room and receive a competitive salary and startup package. For information concerning this position, the Biological Sciences department and university and community resources, please visit our website at **www.udel.edu/bio**.

Please submit a description of research interests, curriculum vitae, and the names of three references with contact information through our website at **http://www.udel.edu/bio/news/facultysearch/** or to Dr. Randall Duncan, Chair, Molecular Physiology Search Committee, Department of Biological Sciences, University of Delaware, Newark, DE 19716-1590. Application deadline is November 15, 2006.

The UNIVERSITY OF DELAWARE is an Equal Opportunity Employer which encourages applications from Minority Group Members and Women.

Faculty Positions in Molecular Biology

The Molecular Biology Program of the Sloan-Kettering Institute, Memorial Sloan-Kettering Cancer Center (www.ski.edu), has initiated a faculty search at the Assistant Member level (equivalent to Assistant Professor). We are interested in outstanding individuals who have demonstrated records of significant accomplishment and the potential to make noteworthy contributions to the biological sciences as independent investigators. Successful applicants will have research interests that move the Program into exciting new areas that complement and enhance our existing strengths in the areas of maintenance of genomic integrity, regulation of the cell cycle, and regulation of gene expression. Faculty will be eligible to hold appointments in the newly established Gerstner Graduate School of Biomedical Sciences, as well as the Weill Graduate School of Medical Sciences of Cornell University.

Candidates should e-mail their application in PDF format to **molbio@mskcc.org** by November 1, 2006. The application should include a CV, a description of past research, a description of proposed research, and copies of three representative publications. Candidates should arrange to have three signed letters of reference sent by email to **molbio@mskcc.org** or by regular mail to **Dr. Kenneth Marians, c/o Steven Cappiello, Box 193, Memorial Sloan-Kettering Cancer Center, 1275 York Avenue, New York, New York 10021.** The letters should arrive by November 1, 2006. Inquiries may be sent to Mr. Cappiello at **molbio@mskcc.org** or to **Dr. Kenneth Marians, Chair, Molecular Biology Program, kmarians@sloankettering.edu.** Memorial Sloan-Kettering is an Equal Opportunity Employer. Smoke-free environment.

Memorial Sloan-Kettering Cancer Center
The Best Cancer Care. Anywhere.
www.mskcc.org

Eastern Virginia Medical School

Professor and Head
Women's Health and Infant Development
Research Center

Eastern Virginia Medical School is making a major commitment to strengthen, expand, and integrate the academic enterprise in basic and clinical research. Our goal is to develop an academic culture which places EVMS at the forefront in biomedical science, education, and health delivery. EVMS and affiliated medical centers, e.g. Sentara Healthcare and Children's Hospital of The King's Daughters, are the primary medical school and health delivery institutions in the Hampton Roads-Mid Atlantic region. EVMS enrolls over 110 new medical students each year and offers graduate programs in the biomedical sciences and public health. The Hampton Roads-Mid Atlantic region offers exceptional education, recreation, entertainment and housing opportunities. During the oncoming year EVMS will undergo an aggressive recruiting effort to develop 4 principal focus areas of research excellence: Heart/ Cardiovascular, Diabetes/Metabolic Diseases; Women's Health/Infant Development and Cancer Biology/Infectious Diseases.

As a first step in this process, we solicit applications for Professor and Head of a new Women's Health and Infant Development Research Center at EVMS. The candidate is expected to: (1) have a Ph.D. and/or M.D.; (2) be a nationally recognized and established scientist in the reproductive sciences; (3) have active NIH R01 or comparable cutting-edge extramural research grant support with translational impact; (4) possess outstanding records of scholarly activity; and (5) foster a collaborative research environment in the new Research Center. The specific area of research is open, but potentially may be on fetal-placental development, fetal programming, placental transport/metabolism, or gene targeting/transfer. The new Center Head will hold a primary academic appointment within the Department of Obstetrics and Gynecology and be expected to participate in hiring an Associate Director, foster collaborative research ties, establish a nationally prominent research center and develop an NIH Program Project or Centers Grant in perinatal/reproductive biology. Long-standing nationally acclaimed research programs in non-human primate perinatal endocrinology and reproductive endocrinology exist at EVMS offering superb opportunities for collaboration and growth. The Women's Health and Infant Development Research Center will span across and be comprised of investigators from several academic departments.

Applicants should submit a letter of interest, an abbreviated NIH Bio-Sketch and full CV listing research grant support to Eastern Virginia Medical School, Office of the Dean, Women's Health and Infant Development Research, Lewis Hall, Room 2019, 700 W. Olney Road, Norfolk, VA, 23507, (Email: hubandsb@evms.edu)

EVMS
Eastern Virginia Medical School

EVMS is an Equal Opportunity/Affirmative Action and Drug Free Workplace Employer and encourages applications of women and minorities.

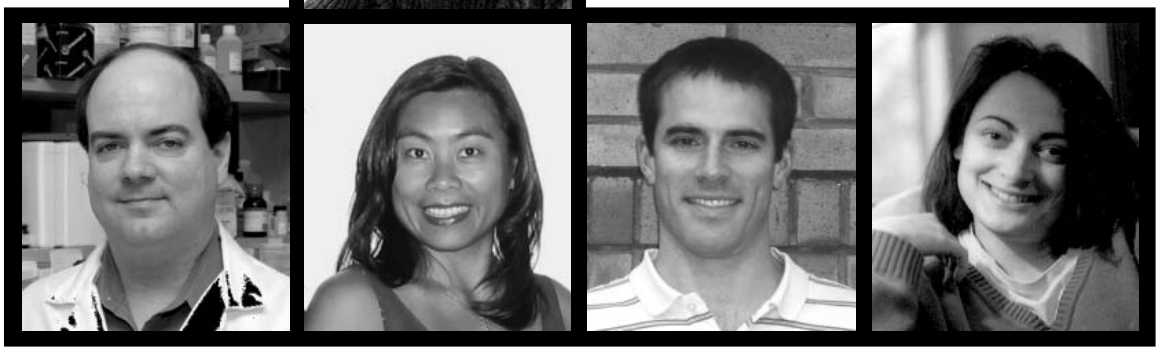
Science Careers Forum

- How long should it take to get my Ph.D.?
- Academia or industry?
- What will make my resume/cv stand out?
- How do I negotiate a raise?

CONNECT WITH EXPERTS

Moderator Dave Jensen
Industry Recruiter

Dave Jensen has over 20 years of experience in human resource consulting and staffing for the biotechnology and pharmaceuticals industry.



Kevin Foley, Ph.D.
Associate Director of In Vivo Pharmacology
Synta Pharmaceuticals

Kevin Foley's research focuses on the discovery and preclinical development of small molecule drugs. He has served as an NIH grant reviewer and as a member of the Scientific Advisory Board for a biotechnology startup company. He has worked in the biotechnology industry since 1998.

Huong Huynh
Program Coordinator, Office of Postdoctoral and Graduate Training
Burnham Institute for Medical Research

Huong Huynh is responsible for professional and career development training of postdoctoral scientists and graduate students. She received her Ph.D. in pharmacology from Loma Linda University.

Andrew Spencer, Ph.D.
Scientist
PDL Biopharma, Inc.

Andy Spencer has a B.S. in chemistry, and a Ph.D. in biochemistry from Michigan State. As a grad student and postdoctoral fellow, Andy spent over ten years in university research labs before moving to the biotechnology industry in 2003.

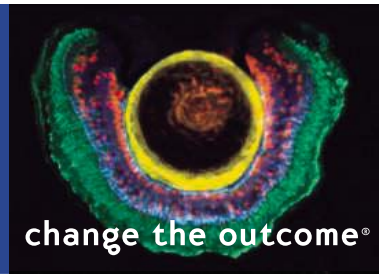
Kelly Suter, Ph.D.
Assistant Professor
University of Louisville School of Medicine

Kelly Suter has a B.S. in chemistry, a B.A. in biology, a Master's in physiology, and a Ph.D. in physiology. She did a postdoc at Colorado State University and was a postdoctoral fellow and research assistant professor at Emory University.

www.sciencecareers.org
and click on *Science* Careers Forum

ScienceCareers.org
We know science AAAS

Faculty Position, Ophthalmology/Human Genetics



Visual Systems Group

Full, Associate or Assistant Professor in Human Genetics

We are seeking a candidate with an outstanding research program in Human Genetics to join the Visual Systems Group. The research arm of the VSG currently consists of Richard Lang, PhD, Rashmi Hegde, PhD, Nadean Brown, PhD, and Tiffany Cook, PhD. The expansion of the VSG is supported by resources from the Division of Pediatric Ophthalmology (Director: Constance West, MD). We are seeking a candidate to complement the expertise of the VSG with a research program investigating the genetic basis of eye or visual system disorders using the strategy of human genetics. This position will be a joint appointment in the Divisions of Pediatric Ophthalmology, and Human Genetics with an academic appointment in the Ophthalmology Department of the University of Cincinnati. Interested candidates should submit a CV, bibliography, and a two-page summary of past research accomplishments and future goals. Please include contact information of three references. Please send to:

Cincinnati Children's® Hospital Medical Center

Visit our web site at
www.cincinnatichildrens.org/research/project/vsg/

Cincinnati Children's Hospital Medical Center and the University of Cincinnati are Affirmative Action Equal Opportunity Employers. Women and minorities are encouraged to apply.

Richard A. Lang, PhD and Constance West, MD
Search Committee Co-Chairs
Cincinnati Children's Hospital Research Foundation
3333 Burnet Avenue ML 7007
Cincinnati, OH 45229-3039

visualsystems@cchmc.org

STEM CELL RESEARCH

MILLER SCHOOL OF MEDICINE
UNIVERSITY OF MIAMI

TENURE-TRACK FACULTY POSITION
DNA METABOLISM / CELL CYCLE

The **Department of Biochemistry and Molecular Biology** invites applications to fill a faculty position in either of the areas listed above. We will consider applicants at any faculty level. For this position, we seek an individual able to develop an independent research program using modern biochemical, molecular biological and biophysical techniques to address a significant biological problem. A generous start-up package and laboratory space are available to support the research program.

Applicants should have a doctoral degree and relevant postdoctoral experience. Demonstrated excellence in research and clear indications of an ability to direct a strong research program are essential. Candidates should also be able to function effectively as a lecturer and in more informal teaching situations at the graduate and undergraduate levels. Interested individuals should send a complete curriculum vitae, at least three letters of recommendation, a brief description of previous research experience and future research plans, and reprints of most significant work to: **Dr. Murray Deutscher, Faculty Search, University of Miami Miller School of Medicine, P.O. Box 016129, Miami, FL 33101-6129**. Review will begin immediately and will continue until the position is filled.

The University of Miami is an Affirmative Action/Equal Opportunity Employer.

RAMAPO COLLEGE OF NEW JERSEY

Ramapo College of New Jersey is located in the beautiful foothills of the Ramapo Valley Mountains, approximately 25 miles northwest of New York City. The School of Theoretical and Applied Science at Ramapo College invites applications for the following tenure-track positions, beginning in Fall 2007.

• ASSISTANT PROFESSOR OF BIOLOGY (Two positions)

MICROBIOLOGY (1):

Responsibilities include teaching microbiology for health sciences, introductory biology for majors and non-majors, participating on committees, in addition to developing a strong research program that involves undergraduate students. The successful candidate is also expected to develop specialty courses in his/her area of interest. Qualified individuals must be committed to excellence in teaching, demonstrated by appropriate teaching experience at the undergraduate level. Interdisciplinary collaborative opportunities are available in biochemistry, bioinformatics, ecology, environmental science, evolutionary biology and molecular biology. ***Requirements:*** Ph.D. is required and postdoctoral experience is preferred. Applications should include reprints of recent publications. Supportive materials in non-electronic format can be sent to ***Dr. Ashley Stuart, Search Committee Chair.***

PLANT BIOLOGY (1):

Seeking a broadly trained plant biologist with an interest in systematics. Applicant will teach plant science courses for biology majors and introductory level biology to majors and non-majors. Candidate is expected to develop specialty courses in his/her specific area of interest. The applicant will have an ongoing and potentially fundable research project that is appropriate for undergraduate participation. ***Requirements:*** Ph.D. or ABD with imminent completion date and a minimum of one year college teaching experience required. ***Dr. William Mitchell, Search Committee Chair.***

• ASSISTANT PROFESSOR OF ENVIRONMENTAL SCIENCE

Teach introductory Environmental Science for majors and non-majors, either Environmental/Occupational Health or Environmental Physics, and develop electives in area of expertise. The successful candidate will be adept in working in an interdisciplinary environment, and have a global perspective. ***Requirements:*** Ph.D. in Biology, Environmental Science, Environmental Physics or related field preferred. ABD with imminent completion date will be considered. Must have applied experience related to environmental and public health and/or energy and energy technologies. Teaching experience at the undergraduate level preferred. Dr. Eric Karlin, Search Committee Chair.

Faculty members are expected to maintain active participation in research, scholarship, college governance, service, academic advisement and professional development activities.

All applications must be completed online at: http://www.ramapojobs.com

Attach vita, cover letter, statement of teaching philosophy, research interests, and a list of three references to your completed application. Since its beginning, Ramapo College has had a mission dedicated to interdisciplinary, intercultural, international and experiential learning. Please tell us how your background, interest and experience can contribute to this mission, as well as to the specific position for which you are applying.

Review of applications will begin immediately and continue until the positions are filled.
Positions offer excellent state benefits. *To request accommodation, call (201) 684-7734.*

RAMAPO COLLEGE OF NEW JERSEY

"New Jersey's Public Liberal Arts College"

Att: Department 25, 505 Ramapo Valley Road
Mahwah, New Jersey 07430

Ramapo College is a member of the Council of Public Liberal Arts Colleges (COPLAC), a national alliance of leading liberal arts colleges in the public sector. EEO/AFFIRMATIVE ACTION.

PICTURE YOURSELF AS A AAAS SCIENCE & TECHNOLOGY POLICY FELLOW!

Advance your career and serve society by plugging the power of science into public policy. Year-long Science & Technology Policy Fellowships offer opportunities in six thematic areas: Congressional • Diplomacy • Energy, Environment, Agriculture & Natural Resources • Global Stewardship • Health, Education, & Human Services • National Defense & Global Security.

Work in Dynamic Washington, D.C.

Since 1973, AAAS Fellows have been applying their expertise to federal decision-making processes that affect people in the U.S. and around the world. A broad range of assignments are available in the U.S. Congress and executive branch agencies.

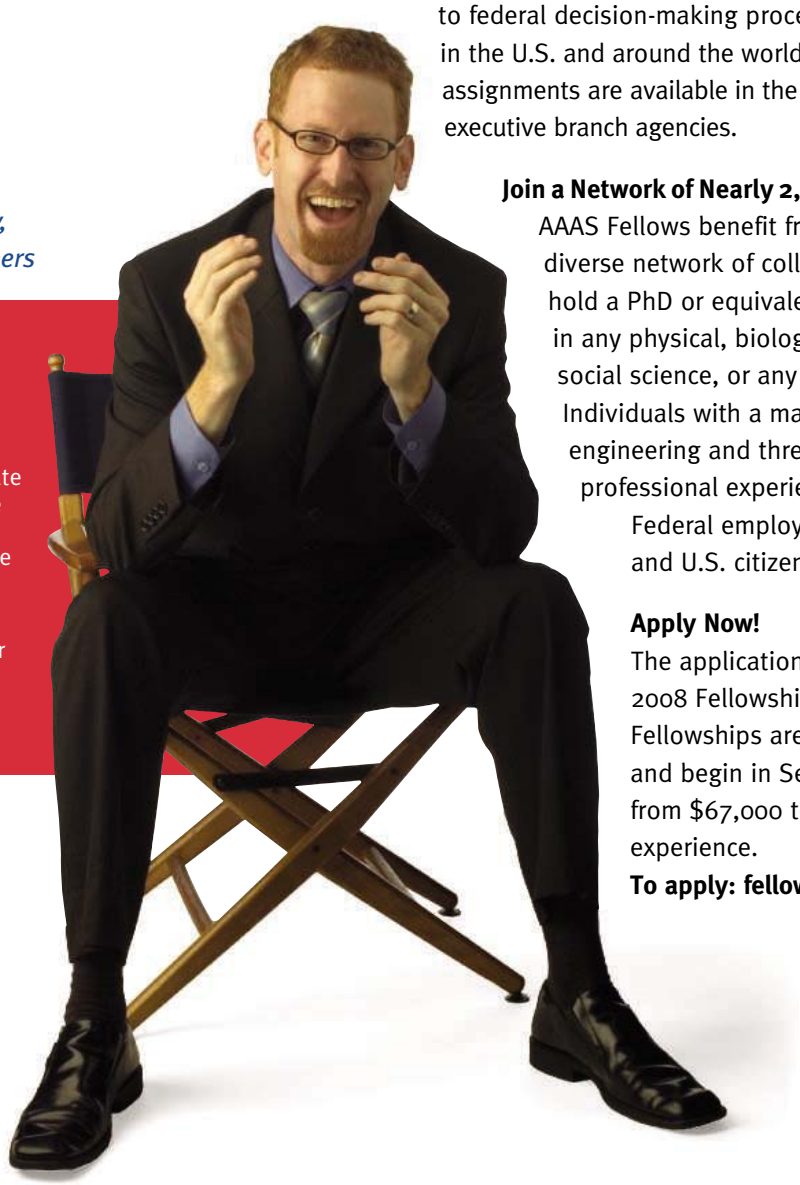
Enhancing Public Policy, Advancing Science Careers

Matt Schmolesky, PhD

Neuroscience, University of Utah.

2004-2006 AAAS Fellow at the U.S. Department of State in the Office of the Science and Technology Adviser to the Secretary and the Office of International Religious Freedom.

Now an assistant professor in the Department of Psychology, Weber State University, Utah.



Join a Network of Nearly 2,000 Fellows.

AAAS Fellows benefit from a growing and diverse network of colleagues. Applicants must hold a PhD or equivalent doctoral-level degree in any physical, biological, medical/health, or social science, or any engineering discipline. Individuals with a master's degree in engineering and three years of post-degree professional experience also may apply. Federal employees are not eligible and U.S. citizenship is required.

Apply Now!

The application deadline for the 2007-2008 Fellowships is 20 December 2006. Fellowships are awarded in the spring and begin in September. Stipends range from $67,000 to $87,000, depending on experience.

To apply: fellowships.aaas.org

AAAS
ADVANCING SCIENCE, SERVING SOCIETY

POSITIONS OPEN

The Biology Division at Kansas State University (KSU) **(website: http://www.ksu.edu/biology)** invites applications for a tenure-track faculty position at the **ASSISTANT PROFESSOR** level, in the general area of animal physiology, beginning fall 2007. Areas of interest, which would complement current research strengths in the Division, include cellular or molecular physiology (e.g., immunophysiology, neuroendocrinology) and physiological ecology (e.g., ecotoxicology, chemical ecology). Applicants should have a Ph.D. in biology, biochemistry, or related discipline; and postdoctoral experience is required. The successful candidate will have demonstrated excellence in research, and must show outstanding promise for developing an independent, extramurally funded research program. A strong commitment to graduate and undergraduate education will also be required, including participation in delivery of our physiology course offerings, as well as a commitment to mentoring of students and to serving a diverse population. Applicants should submit a cover letter, curriculum vitae, brief description of research interests, a statement of teaching experience and philosophy, representative reprints, and have three letters of reference sent to: **Dr. David Rintoul, Chair, Animal Physiology Search Committee, Division of Biology, 116 Ackert Hall, Kansas State University, Manhattan, KS 66506-4901.** Review of applications will begin November 15, 2006, and continue until the position is filled. *KSU is an Equal Opportunity/Affirmative Action Employer, and actively seeks diversity among its employees.*

Engineering and Public Policy at Carnegie Mellon seeks **DOCTORAL STUDENTS** with technical backgrounds to address policy issues in electric power, energy and environmental issues, climate change; information and telecom policy; risk analysis and regulation; R&D, innovation, and development. See **website: http://www.epp.cmu.edu. Victoria Finney, Engineering and Public Policy, Carnegie Mellon, Pittsburgh, PA 15213 U.S.A.**

POSITIONS OPEN

FACULTY POSITION IN BIOCHEMISTRY
Purdue University Department of Chemistry

The Department of Chemistry in the College of Science at Purdue University seeks an outstanding candidate for a tenure-track position beginning August 2007 at the **ASSISTANT PROFESSOR** level. Highly qualified applicants may be eligible for a senior position. Primary consideration will be given to candidates in any area of biochemistry. Candidates of exceptional merit in other areas of chemistry will also be considered. Candidates should have a Ph.D. degree and have demonstrated exceptional ability in research and teaching at the undergraduate and graduate levels.

The College of Science also plans to fill a number of faculty positions in multidisciplinary areas. For more information see the College of Science COALESCE (Cooperative Areas Linking and Extending Science) faculty search webpage at **website: http://www.science.purdue.edu/COALESCE/.** Applicants for COALESCE searches are encouraged to apply online at the website. Applicants to one search may be included in other relevant searches when appropriate.

All applicants for the biochemistry position should apply online at **website: https://applications.science.purdue.edu/chemistry** and include a copy of their curriculum vitae, a summary of planned research, a teaching statement, and arrange to have three letters of recommendation sent to: **Biochemistry Search, Professor Timothy Zwier, Head, Department of Chemistry, Purdue University, 560 Oval Drive, West Lafayette, IN 47907-2084.** The review of applications will begin on November 15, 2006, and continue until the position is filled.

Purdue University is an Equal Access/Equal Opportunity/ Affirmative Action Employer fully committed to achieving a diverse work force. Women and individuals in underrepresented groups are encouraged to apply.

POSITIONS OPEN

BIOLOGY/EDUCATIONAL RESEARCH

Idaho State University **(website: http://www.isu.edu/departments/bios)** invites applications for a tenure-track position at the **ASSISTANT/ASSOCIATE PROFESSOR** level with teaching responsibilities in (a) biology teaching methods, (b) graduate seminars in college biology teaching, and (c) involvement in the Introductory Biology Program. We seek candidates with a doctoral degree in a biological science (with postdoctoral experience preferred) along with demonstrated interest/experience in teacher preparation and conducting research on teaching and learning at the college level. The successful candidate will advise and supervise graduate students conducting research in biological education and will be expected to develop an externally funded research program that complements existing research strengths in the Department. Review of applications will begin 15 November 2006. Send curriculum vitae, statements of teaching and research philosophy, experience, and goals, plus contact information for three references to: **Dr. Jeff Hill, Search Committee Chair, Biological Sciences, Idaho State University, 921 S. 8th Stop 8007, Pocatello, ID 83209-8007.** *Idaho State University is an Equal Opportunity Employer.*

CAREER OPPORTUNITY

This unique program offers the candidate with an earned doctorate in the life sciences the opportunity to obtain the Doctor of Optometry (OD) degree in 27 months (beginning in March of each year). Employment opportunities exist in research, education, industry, and private practice. Contact the **Admissions Office, telephone: 800-824-5526** at **The New England College of Optometry, 424 Beacon Street, Boston, MA 02115.** Additional information at **website: http://www.neco.edu, e-mail: admissions@neco.edu.**

The **Department of Pharmaceutical Chemistry** seeks to hire an **Assistant Professor** interested in problems at the interface of Chemistry and Biology. Of particular interest are those interested in the use of synthetic organic chemistry to solve problems in biology or medicine. The expected appointment is at the level of Assistant Professor (tenure-track), however, senior level appointments in exceptional cases will be considered.

Applicants should have a Ph.D., M.D. or advanced degree with research experience and are expected to establish a dynamic research program. Applicants are also expected to actively participate in graduate training in the Chemistry and Chemical Biology Program or other Programs in Quantitative Biology and in professional school teaching. Applicants will be eligible for membership in the Program in Biological Sciences (PIBS) and other graduate training programs.

UCSF seeks candidates whose experience, teaching, research, or community service has prepared them to contribute to our commitment to diversity and excellence. Please send a curriculum vitae, three letters of reference, a summary of current research (up to 3 pages), and a concise outline of future research (up to 3 pages) by December 1, 2006, to: **Valerie Ohman, Pharmaceutical Chemistry Search Committee, Department of Pharmaceutical Chemistry, University of California San Francisco, 600 16th Street, MC 2280, Genentech Hall, Room N512A, San Francisco, CA 94143-2517.**

UCSF is an Affirmative Action/Equal Opportunity Employer. The University undertakes affirmative action to assure equal employment opportunity for underutilized minorities and women, for persons with disabilities, and for covered veterans.

Monterey Bay Aquarium Research Institute

2007 POSTDOCTORAL FELLOWSHIPS

Founded in 1987 and supported by the David and Lucile Packard Foundation, The Monterey Bay Aquarium Research Institute (MBARI) is a non-profit oceanographic research institute, dedicated to the development of state-of-the-art instrumentation, systems, and methods for scientific research in the oceans. MBARI's research center includes science and engineering laboratories, as well as an operations facility to support our research vessels and oceanographic equipment, including remotely operated and autonomous underwater vehicles. Located in Moss Landing, California, the heart of the nation's largest marine sanctuary, MBARI places a balanced emphasis on science and engineering, with established programs in marine robotics, ocean physics, chemistry, geology, and biology, as well as information management and ocean instrumentation research and development.

MBARI invites applications each year for several postdoctoral fellowships in the fields of biological, chemical, and physical oceanography, marine geology, and ocean engineering. Fellowships may require occasional trips to sea. Awards are typically for two years.

Candidates must complete their Ph.D. degree prior to commencing the two-year appointment between September 2007 and March 2008.

Application deadline: Friday, December 15, 2006

Selected candidates will be contacted in February 2007.

Note: Applicants are encouraged to communicate with potential research sponsors at MBARI **(http://www.mbari.org/about/researchers.html)** for guidance on project feasibility, relevance to ongoing MBARI research, and resource availability.

Application requirements:
1. Curriculum vitae
2. At least three professional letters of recommendation
3. Succinct statement of the applicant's doctoral research
4. Potential research goals at MBARI
5. Supplemental Information online form **(http://www.mbari.org/oed/jobs/forms/postdoc_form.htm)**

Competitive compensation and benefits package.
MBARI considers all applicants for employment without regard to race, color, religion, sex, national origin, disability, or veteran status.

Address your application materials to:
MBARI, Human Resources
Job code: Postdocs-2007
7700 Sandholdt Road, Moss Landing, CA 95039-9644

Submit by e-mail to **jobs_postdocs@mbari.org** (preferred), by mail, or fax to (831) 775-1620.

M B A R I

EOE • MBARI Welcomes Diversity

Keck Graduate Institute
of Applied Life Sciences

Faculty Position in Biomedical Device Engineering

Applications are sought for a faculty member at any level who is dedicated to excellence in interdisciplinary teaching and research in the area of biomedical devices and diagnostics. We seek an individual who can complement our diverse faculty who has expertise and research interests in the integration of biology, engineering, and computational sciences. Of particular interest are entrepreneurial individuals specializing in the development of biomedical devices, sensors, and instrumentation with knowledge of engineering design/development in a regulated environment (FDA 510k or PMA experience). The successful candidate should have PhD in a relevant engineering field and will be expected to pursue externally-funded applied research that could range from single-investigator scientific research to SBIR-type collaborative research and development with industry.

The Keck Graduate Institute *of Applied Life Sciences* is the newest member of the Claremont Colleges and is dedicated to education and research aimed at translating into practice, for the benefit of society, the power and potential of the life sciences. KGI is located 35 miles east of Los Angeles, at the foot of the San Gabriel Mountains. A rich intellectual and cultural environment is provided by the colleges and the surrounding institutions in southern California. The planned start date for this position is Summer or Fall 2007.

Applications should be submitted by **November 30, 2006** and should include a cover letter describing your interests and plans for research and teaching activity with a CV and list of 3 contacts for references to **Prof. James D. Sterling**, Devices Search Committee, Keck Graduate Institute of Applied Life Sciences, **jim_sterling@kgi.edu**. Also see **www.kgi.edu**.

Biology Faculty Positions
Middle Tennessee State University

Middle Tennessee State University, the largest unit in the TBR system with over 23,000 students, invites applications for four tenure-track positions. The department offers baccalaureate and Master's degrees and is committed to quality classroom teaching as well as research and professional service. Ph.D. required by August 2007 start date, post-doctoral experience preferred, rank open. Faculty are expected to direct an extramurally-funded research program involving graduate and undergraduate students, and take part in proposed Ph.D. programs. Application materials must be filed on-line at **http://mtsujobs.mtsu.edu**. Application materials for preliminary review: online employment application, cover letter, and curriculum vita with optional copies of transcripts and statement of teaching philosophy and research program. Application reviews begin **October 29, 2006**. Information on Middle Tennessee State University can be found at **www.mtsu.edu**.

Anatomy and Physiology - Position #103500. Primary teaching responsibility is a two semester human anatomy and physiology course. Expertise in electron microscopy preferred. Area of research interest open. For additional information contact Committee Chair, **Dr. Gore Ervin**, **mervin@mtsu.edu**.

Population Geneticist - Position #103510. The ideal candidate will be fully conversant with modern molecular approaches as applied to the study of ecology and evolution, with preference given to individuals with an interest in plants or microbes (prokaryotic or eukaryotic). Teaching duties may include genetics and freshman biology; potential for upper-division course in area of expertise. For additional information contact Committee Chair, **Dr. Jeff Leblond**, **jleblond@mtsu.edu**.

Vertebrate Biologist - Position #103160. Primary teaching may include introductory biology and mammalogy or ornithology. Research specialty open. For additional information contact Committee Chair, **Dr. George Benz**, **gbenz@mtsu.edu**.

Vertebrate Ecotoxicologist - Position #103580. Teaching responsibilities may include introductory biology and an upper-division/graduate course in area of specialty. Ability to teach upper division courses in ecology and/or biostatistics is desirable. For additional information contact Committee Chair, **Dr. Frank Bailey**, **fcbailey@mtsu.edu**.

EO/AA/ADA Employer.

POSITIONS OPEN

LEONARD AND MADLYN ABRAMSON ENDOWED CHAIR
Department of Pharmaceutical Sciences
Tenure Track, Begin July 1, 2007

The Philadelphia College of Pharmacy, the foundational College of University of the Sciences in Philadelphia, invites applications for the Leonard and Madlyn Abramson Endowed Chair, a tenure-track faculty position in the Department of Pharmaceutical Sciences, to begin on or about July 1, 2007. The successful candidate must have a Ph.D. in an area relevant to pharmaceutical sciences, a well-established research program complementing areas existing in the Department, and a strong record of extramural funding. Participation in teaching in an area of pharmaceutical sciences at the graduate, undergraduate, and professional levels is expected.

The Department consists of 13 tenure-track faculty, five staff members, and approximately 165 undergraduate majors in the B.S. programs in pharmacology/toxicology and pharmaceutical sciences, as well as approximately 55 students in the graduate programs. The Department has a state-of-the-art, Association for Assessment and Accreditation of Laboratory Animal Care-accredited, fully staffed vivarium, tissue culture facilities, and a wide array of analytical instruments including high performance liquid chromatography, liquid chromatography/mass spectrometry, flow cytometer, absorbance and fluorescent plate readers, image analyzers and scanning electron microscope. In addition, the Department has a fully equipped manufacturing/industrial pharmacy laboratory. The new McNeil Science and Technology Center completed in August 2006 provides increased space for research and/or teaching in line with the University's strategic imperative to increase research. There is also a variety of research equipment such as fluorescence spectrophotometer, infrared spectrometer, nuclear magnetic resonance, and confocal microscope which are housed in the Departments of Chemistry and Biology.

Interested parties are encouraged to consult our **website: http://www.usip.edu** for additional information. Each candidate should submit curriculum vitae, letter of application that addresses research interests and teaching experience, and contact information for at least three references as soon as possible to: **Ruy Tchao, Ph.D., Chair, Faculty Search Committee, University of the Sciences in Philadelphia, 600 S. 43rd Street, Philadelphia, PA 19104, e-mail: r.tchao@usip.edu,** and **telephone: 215-596-8978.** Application review will begin immediately; applications will be accepted until the position is filled.

Equal Opportunity/Affirmative Action Employer.

ASSISTANT/ASSOCIATE/FULL PROFESSOR
Department of Biological Sciences
Evolutionary Genetics

The Department of Biological Sciences at Louisiana State University **(website: http://www.biology.lsu.edu)** invites applications for a Tenure-Track or Tenured position in the area of evolutionary genetics. We are especially interested in those whose research interests bridge departmental strengths in ecology and evolution. Required qualifications: Ph.D. or equivalent degree in a biological science or related field. Additional qualification desired: postdoctoral experience. Responsibilities: develops a strong, extramurally funded research program; contributes to teaching and advising at the graduate and undergraduate levels. Review of applications will begin December 1, 2006, and will continue until candidate is selected. We encourage applications from women and minorities. *An offer of employment is contingent on a satisfactory pre-employment background check.* Send curriculum vitae (including e-mail address), statements of research and teaching interests, three letters of recommendation, and no more than three representative publications to: **Dr. Michael Hellberg, Chair, Evolutionary Genetics Search, Department of Biological Sciences, 202 Life Sciences Building, Louisiana State University, Reference 021646, Baton Rouge, LA 70803.** *LSU is an Equal Opportunity/Equal Access Employer.*

POSITIONS OPEN

FACULTY POSITIONS in MOLECULAR, CELLULAR, and DEVELOPMENTAL BIOLOGY
University of Michigan

The Department of Molecular, Cellular, and Developmental Biology at the University of Michigan solicits applications for faculty positions in two broad areas:

(1) Molecular biology. We seek individuals who use biochemical, genetic, cell biological, or structural approaches to address fundamental questions in biology using model organisms including microbes. One of the areas of special interest is plant biochemistry; another is protein folding.

(2) Neurobiology, endocrinology, or developmental biology. We seek individuals who use molecular and genetic approaches to address fundamental questions concerning the function of animal regulatory systems or metazoan development. Individuals with research programs focused on nonmammalian vertebrate or invertebrate model systems are preferred.

We anticipate hiring at the **ASSISTANT PROFESSOR** level, but appointment at a more senior level is possible for applicants with suitable experience. Successful candidates will be expected to establish a vigorous, extramurally funded research program and to be involved in instruction of both undergraduate and graduate students.

To apply, candidate should send a cover letter indicating whether he/she is a candidate for the molecular biology search or the neurobiology, endocrinology, or developmental biology search, curriculum vitae, copies of reprints, brief summaries of recent research accomplishments, a statement of future research plans, and a statement of teaching interests and philosophy. Senior candidates should also provide evidence of teaching excellence. Candidates for appointment as an Assistant Professor should have at least three letters of reference sent immediately to the Department. All materials should be sent to: **Search Committee, Department of Molecular, Cellular, and Developmental Biology, University of Michigan, 830 N. University Avenue, Ann Arbor, MI 48109-1048** or submitted via **e-mail: mcdb-search@umich.edu.** Applications and letters of reference should be received by November 3, 2006.

The University of Michigan is an Affirmative Action/Equal Opportunity Employer. Women and minorities are encouraged to apply. The University is supportive of the needs of dual-career couples.

The Department of Chemistry and Biochemistry at the University of Colorado at Boulder invites applications for a tenure-track faculty position at the **ASSISTANT PROFESSOR** level in analytical/environmental chemistry with a focus area of analytical spectroscopy. The successful candidate will teach undergraduate and graduate courses including general chemistry, instrumental analysis, and analytical spectroscopy. The candidate will be expected to develop a dynamic research program in the general area of analytical spectroscopy. Subdisciplines of particular interest to the Department include instrument development and applications of spectroscopy to environmental chemistry, atmospheric chemistry, and bioanalytical chemistry. A Ph.D. is required and postdoctoral experience is preferred.

It is anticipated that the position will be filled at the Assistant Professor level, but outstanding candidates at a more senior level will also be considered. Applicants should submit curriculum vitae, graduate transcripts, a description of the proposed research and arrange to have three letters of recommendation sent to:

Analytical Spectroscopy Search Committee Chair
Department of Chemistry and Biochemistry
University of Colorado
215 UCB
Boulder, CO 80309-0215

Review of applications will begin on October 15, 2006, and will continue until the position is filled. *The University of Colorado at Boulder is committed to diversity and equality in education and employment.*

POSITIONS OPEN

TWO TENURE-TRACK POSITIONS IN CELL/MOLECULAR BIOLOGY
University of Toledo

The Department of Biological Sciences at the University of Toledo is seeking to fill two tenure-track **ASSISTANT PROFESSOR** faculty positions as part of a major hiring initiative. Departmental research strengths include cellular immunology, cancer biology, nematode molecular biology, molecular neuroscience and plant biology, and other departments in the College of Medicine on the nearby Health Science Campus of the University complement these areas. The new positions will enhance existing research strengths. Facilities include a modern research complex with state-of the-art laboratories and outstanding instrumentation centers with plans underway for a new science building. Applicants must have a Ph.D. and postdoctoral experience. Successful candidates should have or will be expected to develop an externally funded research program and will participate in undergraduate and graduate instruction. The Department offers the B.S., M.S., and Ph.D. degrees. Additional information is available on the Departmental **website: http://www.biosciences.utoledo.edu.** Salary and startup funds are competitive. Review of applications will begin October 20, 2006, and continue until the positions are filled. The starting date for these positions will be August 2007. Interested candidates should send a letter of application, curriculum vitae, statements of teaching and research interests, and arrange to have three letters of recommendation sent to: **Chair, Faculty Search Committee, Department of Biological Sciences, M.S. 601, University of Toledo, Toledo, OH 43606-3390.** E-mail inquiries may be directed to **e-mail: john.plenefisch@utoledo.edu** or **e-mail: patricia.komuniecki@utoledo.edu.**

Qualified women and minorities are encouraged to apply. The University of Toledo is an Affirmative Action/Equal Opportunity Employer Minorities/Females/Persons with Disabilities/Veterans.

FACULTY POSITION
ASSISTANT PROFESSOR (TENURE TRACK)
Department of Psychiatry

The Department of Psychiatry at the Uniformed Services University of the Health Sciences, Bethesda, Maryland, is seeking to fill an Assistant Professor, tenure-track, teaching and research position with particular emphasis on biological psychiatry. The Department is comprised of 20 full-time faculty and has active research interests in the neurobiology and behavior of stress, post-traumatic stress disorder, anxiety, depression, and substance abuse. The successful candidate will be responsible for developing a funded research program and will participate in medical student and resident education and clinical care. Individuals who hold an M.D., have completed an approved psychiatric residency and are Board-eligible/certified are invited to apply. Send curriculum vitae, description of current and anticipated research interests, and the names and addresses of four references to: **Robert J. Ursano, M.D., Chairman, Department of Psychiatry, Uniformed Services University, 4301 Jones Bridge Road, Bethesda, MD 20814 (e-mail: rursano@usuhs.mil).** Review of applications is ongoing. *The University is an Affirmative Action/Equal Opportunity Employer.*

DIRECTOR/SCIENTIST
High-Throughput Screening Laboratory

The University of Kansas (KU) is seeking a highly motivated individual to manage its High-Throughput Screening (HTS) facility. The Director will have the scientific, administrative, and fiduciary oversight over the facility and conduct collaborative research with groups interested in HTS. A Ph.D. or equivalent degree is required. Salary is dependent on experience. For more detailed information on qualifications and how to apply see **website: https://jobs.ku.edu.** Search for 00066632. Review of applications will begin November 1, 2006. *Equal Opportunity/Affirmative Action Employer.*

POSITIONS OPEN

TENURE-TRACK ASSISTANT PROFESSOR OF BIOLOGICAL AND BIOMEDICAL SCIENCES

The Department of Biological Sciences **(website: http://www.isu.edu/departments/bios)** at Idaho State University seeks applicants for a tenure-track position to begin August 2007. Teaching responsibilities include medical histology, oral histology and embryology, and a course in the candidate's expertise. Applicants must have a doctorate (postdoctoral experience preferred). The successful candidate is expected to mentor graduate and undergraduate students and establish a rigorous, extramurally funded research program that complements and enhances our current biomedical research emphases. Salary will be commensurate with training and experience, and a competitive startup package will be provided. Review of applications begins 15 January 2007, and will continue until filled. To apply, send cover letter, curriculum vitae, statement of teaching philosophy and research program, and contact information for three references to: **Dr. Curt Anderson, Chair, Histology and Embryology Search Committee, Department of Biological Sciences, 921 South 8th Avenue, Stop 8007, Idaho State University, Pocatello, ID 83209-8007 U.S.A.** *Idaho State University is an Equal Opportunity Employer. Women and members of minority groups are encouraged to apply.*

ASSISTANT/ASSOCIATE/FULL PROFESSOR

The Department of Physiological Sciences, College of Veterinary Medicine, Oklahoma State University (OSU), invites applications for a tenure-track position at the rank of Assistant Professor, Associate Professor, or Professor. Applicants with a Ph.D. in pharmacology, toxicology, or physiology are encouraged to apply, and applicants with both D.V.M. and Ph.D. will be strongly considered. Primary responsibilities include development of an extramurally supported research program and participation in both the veterinary medicine professional curriculum and biomedical sciences graduate program. Candidates whose scholarly achievements meet the selection criteria will receive consideration for the Lundberg-Keinlen Endowed Professorship in Biomedical Research. Interested individuals should submit an application including curriculum vitae, statement of professional goals, and names of three references to: **Dr. Carey Pope, Head, Department of Physiological Sciences, 264 McElroy Hall, Center for Veterinary Health Sciences, Stillwater, OK 74078 (telephone: 405-744-6257, e-mail: carey.pope@okstate.edu).** To ensure full consideration, applications should be received by December 1, 2006. Review of applications will continue until a suitable candidate is identified.

OSU is an Equal Opportunity/Affirmative Action Employer that encourages applications from members of minority groups.

ASSISTANT/ASSOCIATE/FULL PROFESSOR SYSTEMS PHYSIOLOGIST
Department of Biological Sciences

The Department of Biological Sciences at Louisiana State University (LSU) **(website: http://www.biology.lsu.edu)** invites applications for a Tenure-Track or Tenured position in systems physiology. Required qualifications: Ph.D. or equivalent degree in biological sciences or a related field; postdoctoral experience; record of creative and significant research in any area of experimental animal physiology (non-mammalian model systems preferred). Responsibilities: develops a vigorous, extramurally funded research program; contributes to undergraduate and graduate teaching. *An offer of employment is contingent on a satisfactory pre-employment background check.* Review of applications will begin December 1, 2006, and will continue until candidate is selected. Send curriculum vitae (including e-mail address), statement of research and teaching interests, three letters of recommendation and reprints of key publications to: **Systems Physiology Search Committee, Department of Biological Sciences, 202 Life Sciences Building, Louisiana State University, Reference 024645, Baton Rouge, LA 70803.** *LSU is an Equal Opportunity/Equal Access Employer. We encourage applications from women and minorities.*

Harvard Stem Cell Institute (HSCI) is recruiting a **DIRECTOR OF TRANSLATIONAL MEDICINE** (DTM) and **CHIEF SCIENTIFIC OFFICER** of the Technology Accelerator Fund to be responsible for design and execution of biological and chemical screens for therapeutic agents, which are a critical part of the HSCI's mission. The DTM will build two research teams, one focused on collaborative research with HSCI members and another focused on the development of therapeutics. The DTM will be a senior member of the HSCI executive team and help develop strategy, review disease programs, and raise funds for research; the DTM will also work with faculty to oversee graduate students and Postdoctoral Fellows involved in experimental design and screening execution with the collaborative team. The DTM will be responsible for working with foundations and corporations to develop and fund specific research projects. The DTM will be expected to participate in the educational mission of the HSCI. The DTM will chair the therapeutics committee, which will set priorities, review screens, and monitor results. As Chief Scientific Officer of the Technology Accelerator Fund, the candidate will report to the Senior Associate Provost for Technology Development, with responsibility for managing a portfolio of life science R&D projects supported and operated by the Harvard University Technology Accelerator Fund, and will: provide leadership in managing Accelerator Fund projects; play a key role in defining decision points, timelines and resources in alignment with R&D goals and objectives; provide input to the prioritization process of each project and participate in other key strategic decision-making processes; track and control project expenditures to stay within budget, eliminate or minimize barriers to progress. We are seeking an M.D. or Ph.D. with extensive experience in academia and industry, with demonstrable leadership of successful research teams. Experience and proven success with designing and implementing biological screens for therapeutic development and in leading and managing preclinical drug development projects are essential, as well as strong interpersonal skills for bridging between multiple stakeholders and achieving consensus with respect to project performance, timelines, and resource utilization. Candidates should send a letter of interest, curriculum vitae, and three letters of support to: **Dr. David Scadden, c/o Christina Shambaugh (e-mail: cpasker@partners.org).**

FACULTY POSITION
Optical Spectroscopy

The Department of Chemistry and Biochemistry at the University of Arkansas is seeking an outstanding scientist for a tenure-track faculty position associated with the NIH National Center for Research Resources Center for Protein Structure and Function **(website: http://www.uark.edu/chemistry).** We seek candidates with expertise in the use of modern optical spectroscopy for investigating proteins and biomolecules, including single-molecule methods or other novel approaches. The Department has state-of-the-art core facilities in nuclear magnetic resonance spectroscopy, protein and small molecule X-ray crystallography, mass spectrometry, and high-throughput synthesis. Collaborative, multidisciplinary research projects are encouraged. Successful candidates must have a Ph.D. and will be expected to establish a nationally funded research program, and teach effectively at the graduate and undergraduate levels. Review of completed applications will begin on November 1, 2006, and continue until the position is filled. Curriculum vitae, statements of research plans, and teaching interests, and three letters of recommendation should be sent to: **Dr. Frank Millett, Faculty Search Committee, Department of Chemistry and Biochemistry, University of Arkansas, Fayetteville, AR 72701 (website: http://millett@uark.edu).** Women and minority candidates are strongly encouraged to apply. *The University of Arkansas is an Affirmative Action/Equal Opportunity Employer. All applicants are subject to public disclosure under the Arkansas Freedom of Information Act, and persons hired must have proof of legal authority to work in the United States.*

TENURE-TRACK BIOCHEMIST and ORGANIC CHEMIST. Hamilton College invites applications for two tenure-track, open-rank positions, beginning July 2007. An entry-level appointment is likely, but appointment at higher ranks is possible and senior scholars are encouraged to apply. Applicants must be committed to undergraduate education and demonstrate excellence, or the potential for excellence, in teaching and research with undergraduates. Special consideration will be given to candidates with biophysical, bioorganic, bioanalytical, or green chemistry research programs. Teaching responsibilities may include general chemistry, research methods, and other courses in the candidate's areas of expertise. The successful candidate will be expected to guide student research during the summer and advise the required senior project during the academic year. Ph.D. and postdoctoral experience required. Excellent startup support. You will join a group of committed teacher/scholars who insist on excellence in teaching and have a passion for educating students through one-on-one mentoring of research. We work in a supportive collegial environment with superb administrative support, in a new state-of-the-art facility. Further information about Hamilton and the Department can be found at **website:http://www.chem.hamilton.edu.** Please send curriculum vitae, undergraduate and graduate transcripts, statements describing teaching and research interests, and arrange for three letters of recommendation to be sent by October 30, 2006, to: **Karen S. Brewer, Chair, Department of Chemistry, Hamilton College, 198 College Hill Road, Clinton, NY 13323.** *Hamilton College is an Equal Opportunity, Affirmative Action Employer and is committed to diversity in all areas of the campus community. Hamilton provides domestic partner benefits.*

ASSISTANT PROFESSOR
Genetics of Neural Systems and Behavior

We invite applications for a tenure-track Assistant Professor appointment in the Division of Biology at the California Institute of Technology. We are seeking highly qualified candidates who are committed to a career in research and teaching. The applicant should conduct research at the interface of molecular biology and systems neuroscience aimed at understanding neural circuits and the control of behavior. We encourage applications from individuals who may, but need not, work on conventional genetic model organisms, either vertebrate or invertebrate.

Appointment is contingent upon completion of Ph.D. Submit online a brief cover letter, curriculum vitae, relevant publications, and a description of proposed research. Arrange to have at least three letters of recommendation sent by **e-mail: genetics-search@caltech.edu.** Please visit **website: http://www.biology.caltech.edu/Positions** for application submission. *The California Institute of Technology is an Equal Opportunity/Affirmative Action Employer. Women, minorities, veterans, and disabled persons are encouraged to apply.*

RESEARCH ASSOCIATE (ASSISTANT PROFESSOR) POSITION available in the Department of Neurology to study the mechanisms of neural protection from brain activity. We are interested in tissue, cellular, and molecular bases by which brain becomes more resilient to disease through neural activity. Candidates must have a doctorate in biological sciences, molecular biology, or a related field with at least four years of experience in experimental neuroscience. Salary will be commensurate with background and experience. Send curriculum vitae, a personal research statement, names of three references, and up to five best publications to **Dr. Richard P. Kraig** as hard copy to: **Department of Neurology, MC2030, University of Chicago, 5841 South Maryland Avenue, Chicago, IL 60637 (fax: 773-702-5175)** or **via e-mail: rkraig@neurology.bsd.uchicago.edu** with Microsoft Word and PDF files as needed. Screening of applications will continue until the position is filled. *The University of Chicago is an Affirmative Action/Equal Opportunity Employer.*

POSITIONS OPEN

DEVELOPMENTAL BIOLOGIST. The Wabash College Biology Department invites applications for a Byron K. Trippet Assistant Professorship in developmental biology. Tenure-track position to start July 1, 2007. Ph.D. and a commitment to excellence in teaching required; teaching experience desirable. Development of a research program involving undergraduates is expected. Primary expertise should be in plant or animal developmental biology. Preference will be given to candidates with background and expertise in one or more of the following areas: molecular biology, developmental genetics, or evolutionary developmental biology. In a two-year cycle, responsibilities include teaching a course in developmental biology, a course in the applicant's special area of expertise, and participating in team-taught general biology courses. Periodic contributions to all-college courses are expected. The Biology Department, with seven full-time faculty positions, encompasses molecular biology, organismal biology, and population biology. The Department is well-equipped for teaching and research in a new science facility. The Byron K. Trippet Assistant Professorship provides a summer research stipend during the first two years along with significant startup funds. Send a letter of application, curriculum vitae, brief statements of teaching philosophy and research interests, undergraduate and graduate transcripts, and three letters of recommendation by December 1, 2006, to: **Dr. Eric J. Wetzel, Chair, Biology Department, Wabash College, P.O. Box 352, Crawfordsville, IN 47933.** No electronic applications accepted. Questions may be directed to **e-mail: wetzele@wabash.edu.** Information about the College and Department is available at **website: http://www.wabash.edu/.** *Wabash College, a liberal arts college committed to the education of undergraduate men, encourages applications from women and minorities. Equal Opportunity Employer.*

ASSISTANT/ASSOCIATE/FULL PROFESOR
Signal Transduction and/or
Modulation of Gene Expression
Department of Biological Sciences/
Biochemistry and Molecular Biology

The Department of Biological Sciences at Louisiana State University (LSU) **(website: http://www.biology.lsu.edu)** invites applications for a Tenure-Track or Tenured position in the broad area of cell signaling. Required qualifications: Ph.D. or equivalent degree in biological sciences or a related field; postdoctoral experience; record of creative and significant research. Additional qualifications desired: expertise in studying signal transduction, modulation of gene expression, and/or protein trafficking in nonmammalian model systems. Responsibilities: develops a vigorous, extramurally funded research program; contributes to undergraduate and graduate teaching. *An offer of employment is contingent on a satisfactory pre-employment background check.* Review of applications will begin December 1, 2006, and continue until candidate is selected. Send curriculum vitae (including e-mail address), statement of research and teaching interests, three letters of recommendation and reprints of key publications to: **Dr. Jackie Stephens, Chair, Biochemistry and Molecular Biology Search Committee, Department of Biological Sciences, 202 Life Sciences Building, Louisiana State University, Reference 000391, Baton Rouge, LA 70803.** *LSU is an Equal Opportunity/Equal Access Employer. We encourage applications from women and minorities.*

University of Southern California in Los Angeles seeks a **RESEARCH ASSOCIATE.** Tasks include: in vivo studies in mice; RNA, DNA isolation; cloning; PCR; Northern/Southern/Western blotting; transfection; footprinting; gel shift; promoter analysis; DNA methylation; cell biology; transgenic mice; immunohistochemistry and protein isolation. Job requires Ph.D./M.D. or equivalent in biology or related science/medical discipline and one year of experience in related field. Submit curriculum vitae with references to **website: https://sjobs.brassring.com/1033/ASP/TG/cim_jobdetail.asp?partnerid=101&siteid=5271&OReq=H10460.**

FACULTY POSITION IN SYSTEMS NEUROPHYSIOLOGY
at University of Southern California

The Section of Neurobiology in the Department of Biological Sciences at the University of Southern California (USC) invites applications for a tenure-track faculty position, at any rank, in the area of systems neurophysiology.

The Section is part of a broad interdisciplinary Neuroscience community at USC, composed of more than 60 faculty conducting research in basic, engineering, and clinical sciences **(website: http://www.usc.edu/programs/neuroscience/).** For consideration, applicants must have a Ph.D., M.D., or equivalent degree and have demonstrated the ability to conduct innovative research into fundamental questions of neural function. Candidates who investigate sensory, motor, or cognitive processing from single cell to network levels are encouraged to apply. Experimental approaches such as imaging, intracellular, or multicellular recording in the whole animal, or a combination of in vivo with in vitro preparations, are of particular interest. Given the interdisciplinary emphasis of neuroscience at USC, a candidate's potential to collaborate with other Experimental and/or Computational Neuroscientists is highly desirable. Applications received before November 30, 2006, will be certain to receive consideration.

Applicants should supply their curriculum vitae, a statement of research interests, and three letters of recommendation to:

Judith Hirsch, Ph.D. c/o Vanessa Clark
University of Southern California
Hedco Neuroscience Building, 120, MC 2520
3641 Watt Way
Los Angeles, CA 90089-2520

The University of Southern California is an Affirmative Action/Equal Opportunity Employer.

BIOLOGY FACULTY POSITION

The University of Redlands invites applications for a Tenure-Track Faculty position in biology. We seek to broaden our existing course and research offerings. The successful candidate will teach both nonmajors and majors as well as involve undergraduates in research. Areas of expertise might include, but are not limited to, invertebrate biology, systems physiology, and computational biology. A Ph.D. (by September 2007), evidence of excellence in undergraduate teaching, and a commitment to undergraduate research are required. Please send letter of application, curriculum vitae, a description of research plans, a statement of teaching philosophy with a list of potential course offerings, and arrange for three letters of recommendation to be sent to: **Chair, Search Committee, Department of Biology, University of Redlands, P.O. Box 3080, Redlands, CA 92373-0999.** Applications received by November 17, 2006, are assured full consideration. Located in an ethnically and culturally diverse region midway between Los Angeles and Palm Springs, the University of Redlands **(website: http://www.redlands.edu)** is a private, selective, liberal arts University enrolling approximately 2,300 undergraduates in the residential College of Arts and Sciences. *The University of Redlands is an Equal Opportunity Employer. We actively seek applications from members of under-represented populations.*

BIOLOGY. The Department of Biology, York University, Toronto, Ontario, Canada, invites applications for two **TENURE-TRACK POSITIONS** in physiology (plant or animal) and molecular and/or cell biology. Details are available at **website: http://www.yorku.ca/acadjobs.** *York University is an Affirmative Action Employer. The Affirmative Action Program can be found on York's* ***website: http://www.yorku.ca/acadjobs*** *or a copy can be obtained by calling the* ***Affirmative Action Office*** *at* ***telephone: 416-736-5713.*** *All qualified candidates are encouraged to apply; however, Canadian citizens and permanent residents will be given priority.*

FACULTY POSITION IN BIOCHEMISTRY
University of California, San Diego

The Department of Chemistry and Biochemistry of University of California, San Diego (UCSD), **(website: http://chem.ucsd.edu)** invites applications for a tenure-track/tenured faculty position in biochemistry. Candidates must have a Ph.D. and a demonstrated ability for creative research and teaching at the undergraduate and graduate levels. The Department will consider applicants in all areas of cellular biochemistry and chemical biology in eukaryotic systems with particular emphasis on membrane biology, protein degradation, DNA repair and replication, molecular immunology and splicing. Salary commensurate with qualifications and based on University of California pay scale. Candidates should send curriculum vitae, list of publications, reprints of up to five representative papers, and a summary of research plans to: **Chair, Biochemistry Search Committee, 4-7918, University of California, San Diego, Department of Chemistry and Biochemistry, Mail Code 0332, La Jolla, CA 92093-0332.** Candidates should also arrange to have three letters of reference sent under separate cover. The deadline for applications is November 1, 2006, but until position is filled, all applications received will be assured full consideration. *UCSD is an Equal Opportunity/Affirmative Action Employer with a strong institutional commitment to the achievement of diversity among its faculty and staff.*

FACULTY POSITION
Microbiology/Virology

The Department of Microbiology and Immunology at Des Moines University, Osteopathic Medical Center invites applicants for a tenure-track position at the level of **ASSISTANT or ASSOCIATE PROFESSOR.** The successful candidate must possess the knowledge and skills necessary to participate in the teaching of medical virology to medical and health professions students. In addition, it is expected that the individual develop an innovative and extramurally funded research program using contemporary approaches to study host-pathogen interaction. Applicants should have a Ph.D. and relevant postdoctoral experience. Rank and salary are commensurate with training and experience. Applicants should e-mail curriculum vitae, a concise statement of teaching and research interests, and the names of three professional references to the **Microbiology Faculty Search Committee** at **e-mail: employment@dmu.edu.** Visit **website: http://www.dmu.edu.** For full consideration applications should be received by January 31, 2007. *Des Moines University is an Equal Opportunity Employer.*

PHARMACOLOGY EDUCATOR. The Department of Pharmacology and Experimental Neurosciences at the University of Nebraska Medical Center (UNMC) seeks applicants for an open track, tenure leading position. A Ph.D. in pharmacology or related discipline and evidence of excellence in teaching medical and allied health students are required. The successful candidate will be broadly trained in pharmacology with outstanding communication, interpersonal, and administrative skills. Opportunities to instruct in upper level pharmacology graduate courses and engage in research activities will be available. Interested individuals are asked to submit curriculum vitae, the names of three references, and a statement of education philosophy to: **Dr. Terry Hexum, 985800 Nebraska Medical Center, Omaha, NE 68198-5800 (e-mail: pharmeducator@unmc.edu)** on or before December 31, 2006. Applications will be reviewed starting January 1, 2007, and the review process will continue until the position is filled. The anticipated start date is July 1, 2007. *UNMC is an Equal Opportunity/Affirmative Action Employer. Women and minorities are encouraged to apply.*

POSITIONS OPEN

DEVELOPMENTAL BIOLOGY
Tenure-Track Position
Bowdoin College

The Biology Department at Bowdoin College invites applications for a tenure-track position in developmental biology at the **ASSISTANT PROFESSOR** level beginning fall 2007. We are seeking candidates who will demonstrate excellence in both teaching and research. Postdoctoral experience preferred. Typical teaching responsibilities each year include one laboratory course in developmental biology (with a laboratory instructor), one course at the nonmajors or introductory biology level, and one advanced course in one's area of research. The successful applicant is expected to pursue an active research program that involves undergraduates.

Review of applications will begin November 1, 2006, and will continue until the position is filled. Please send curriculum vitae and a description of your research interests and teaching philosophy, and arrange to have three letters of reference sent to: **Search Committee Chair, Biology Department, 6500 College Station, Bowdoin College, Brunswick, ME 04011-8465.** For further information about the College, the Department, and the program, please see our **website: http://academic.bowdoin.edu/biology/.**

Bowdoin College is committed to equality through Affirmative Action and is an Equal Opportunity Employer. We encourage inquiries from candidates who will enrich and contribute to the cultural and ethnic diversity of our College. Bowdoin College does not discriminate on the basis of age, race, creed, color, religion, marital status, gender, sexual orientation, veteran status, national origin, or disability status in employment, or in our education programs.

ANALYTICAL SCIENTIST

Responsible for supporting all aspects of drug development within Sepracor's Chemistry R&D Department.

Requires a Master's degree in analytical chemistry, or a related field or equivalent. Also requires two years of experience in the job offered or two years of pharmaceutical analysis experience. An individual in this position must have well-demonstrated ability in the development and validation of analytical methods for the testing and characterization of pharmaceutical substances and products. Analytical skills should include: high performance liquid chromatography (isocratic, gradient), gas chromatography, thermal analysis, infrared spectroscopy, ultraviolet and visible spectroscopy, mass spectrometry, titrimetry, chiral analysis, and general wet and compendial techniques; must be proficient in the use of Millennium/Empower, Chemstation, Omnic, Pyris, TA Universal, Lab X, Microsoft Word and Excel software packages. Also required is knowledge of good laboratory practice/good manufacturing practice requirements. An individual in this position must have strong communication skills, demonstrated teamwork skills, and be willing and able to work in a multidisciplinary environment. In addition, an individual in this position must have demonstrated problem solving and data analysis abilities, instrument troubleshooting and maintenance, demonstrated leadership, written and verbal skills, and good organizational skills.

Please submit your resume and salary requirements to **e-mail: resumesatsepracor@sepracor.com** or to: **Eileen Rivera** at **Sepracor, Inc., 84 Waterford Drive, Marlborough, MA 01752.** *Equal Opportunity Employer.*

POSTDOCTORAL FELLOWSHIP IN NANOTECHNOLOGY. Burnham Institute for Medical Research, an independent, nonprofit, public-benefit basic research organization, located in La Jolla, California, is seeking Postdoctoral Associates. Projects involve (1) linking engineered proteins to nanodevices to endow them with biological control, (2) creating synthetic HDL to clear atherosclerotic plaque, (3) engineering nanoparticles with antiplatelet properties. Strong skills in protein engineering, construction of peptide amphiphiles, or platelet and plaque biology are required. Publication record is strongly encouraged. To apply, please send curriculum vitae and three references via **e-mail: jameslee@burnham.org. Website: http://www.burnham.org.**

POSITIONS OPEN

CONSERVATION BIOLOGIST
TENURE-TRACK ASSISTANT PROFESSOR
Department of Ecology and Evolutionary Biology and Center for Environmental Studies
Brown University

The Department of Ecology and Evolutionary Biology and the Center for Environmental Studies at Brown University seek a Conservation Biologist to join its faculty. We particularly welcome applicants with interdisciplinary interests that would complement existing faculty strengths in ecosystem and community ecology, population genetics and evolutionary biology, environmental policy, environmental health as well as ongoing University initiatives in environmental change, the social sciences and genomics and our institutional partnership with the Marine Biological Laboratory at Woods Hole. Requirements include a Ph.D., a strong record of research excellence, and potential for excellence in teaching. Candidates whose scholarly work links conservation science and theory with pressing policy and management issues are encouraged to apply. The new hire will be expected to develop a strong, externally funded research program, teach courses such as conservation biology, and contribute to undergraduate and graduate training.

To apply, please send curriculum vitae, statement of research and teaching interests, representative publications, and arrange to have three letters of recommendation sent to: **Mark Bertness, Chair, Conservation Biology Search Committee, P.O. Box G, Brown University, Providence, RI 02912.** For further inquiries, please contact **e-mail: mark_bertness@brown.edu.** Applications will be reviewed starting 30 November 2006, and accepted until the position is filled. The start date for this position is 1 July 2007, or as soon thereafter as is feasible. *Brown University is an Equal Employment Opportunity/Affirmative Action Employer.*

EXECUTIVE VICE PRESIDENT

National Disease Research Interchange (NDRI), a not-for-profit company providing scientists with human biomaterials for research, invites applications for Executive Vice President. The Executive Vice President will possess strong financial and organizational competencies with a minimum of ten years of experience working in a scientific environment requiring business management skills. Requires demonstrated success in negotiating sponsored research agreements, supervising technology joint ventures, and evaluating new science and technology.

Qualified candidates with an advanced degree in medicine or a Ph.D. in the biological sciences or medicine in molecular biology, immunology, genetics, pathology, or a related field are expected to have submitted successful grant applications to NIH and be familiar with NIH reporting requirements and leadership. Superior communication skills required. Computer expertise to include advanced spreadsheet, database, and reporting skills. Must have excellent analytic, writing, and presentation skills. An energetic team player committed to organizational growth and identification of new opportunities is required. Competitive salary and excellent benefits. E-mail curriculum vitae to **e-mail: smcgovern@ndriresource.org,** or fax to **S. McGovern** at **fax: 215-557-7154,** or mail to:

Attn: S. McGovern
1628 John F. Kennedy Boulevard
8th Floor, 8 Penn Center
Philadelphia, PA 19103

FACULTY POSITIONS, BIOLOGY

Two tenure-track, **ASSISTANT PROFESSOR** positions: (1) **PHYSIOLOGIST,** (2) **AVIAN BIOLOGIST.** Both must participate in graduate programs, teach undergraduate and graduate courses, and develop externally funded research programs; available fall 2007; Department of Biology, Boise State University. Review of applications begins 13 November 2006. Position description and application procedures are at **website: http://hrs.boisestate.edu/joblistings/faculty/.** *Boise State University is an Equal Opportunity Employer/Affirmative Action Employer. Veterans Preferences.*

POSITIONS OPEN

CENTER OF EXCELLENCE DIRECTOR

The Department of Veterans Affairs' Central Texas Veterans Health Care System (CTVHCS) seeks a Director for a new Center of Excellence for Mental Health and Post-traumatic Shock Disorder (PTSD), located at the Waco campus of CTVHCS. The Program is on the cutting edge of implementing psychosocial rehabilitation for the seriously mentally ill, and is a teaching hospital of the Texas A&M University Health Science Center College of Medicine. Candidates must demonstrate a sustained, independent funded research program in PTSD or psychosocial rehabilitation, as well as the vision to successfully guide the Center of Excellence to national prominence. CTVHCS is currently implementing major funding for a new research program in Post-traumatic and Developmental Stress Disorders, and administrative experience in overseeing translational research will be highly regarded. Funding will be immediately available to hire at least two additional experienced investigators and administrative personnel to support the Program, with additional support expected as the Center of Excellence is implemented.

Salary is commensurate with qualifications and experience. *Applicants must be U.S. citizens with a current unrestricted license to practice medicine in the U.S.* or an earned Ph.D. degree. The position offers excellent benefits, low cost of living, and no state income tax.

To apply contact:

Mary Doerfler, Human Resources Specialist (05)
Central Texas Veterans Health Care System
1901 Veterans Memorial Drive, Temple, TX 76504
Telephone: 254-743-0049, fax: 254-743-0007
E-mail: mary.doerfler@med.va.gov

ASSISTANT PROFESSOR, TENURE TRACK, OCCUPATIONAL/ENVIRONMENTAL HYGIENE. The Department of Environmental Health Sciences (ENHS) at the University of South Carolina invites applications from Ph.D.s with research/teaching interests in industrial hygiene, exposure assessment and control, biological monitoring, infection control, and/or toxicogenomics. ENHS offers Master's and doctoral degrees in environmental quality, industrial hygiene, and hazardous materials management. The successful applicant will be expected to contribute to our Accreditation Board for Engineering and Technology-accredited Industrial Hygiene Program, and will collaborate in other program areas. Strong evidence of the ability to develop an extramurally funded research program is desired. Application reviews begin immediately. Applicants should apply online at **website: http://uscjobs.sc.edu,** requisition 042522, and also forward curriculum vitae, statement of professional goals in research and teaching, and contact information for three references to **Dr. C. E. Feigley**, **e-mail: cfeigley@sc.edu.** *The University of South Carolina is an Affirmative Action, Equal Opportunity Employer. Women and minorities are encouraged to apply.*

ENVIRONMENTAL HEALTH, ASSISTANT PROFESSOR/TENURE TRACK. The Graduate School of Public Health, San Diego State University (SDSU), invites applications from individuals with strong background in laboratory or modeling sciences and research interests in one or more of the following areas: exposure assessment, global health, toxicology, environmental monitoring, occupational health, pollutant monitoring. Apply with letter of application, curriculum vitae, and name/contact information of at least five references to: **Searches, Graduate School of Public Health, San Diego State University, 5500 Campanile Drive, San Diego, CA 92182-4126, website: http://publichealth.sdsu.edu.** Application review begins December 1, 2006, and continues until the position is filled. *SDSU is a Title IX Employer and does not discriminate against individuals on the basis of race, religion, national origin, sexual orientation, gender, marital status, age, disability, or veteran status, including veterans of the Vietnam era.*

Stanford University Evolutionary Developmental Biologist Faculty Position

The Department of Biological Sciences at Stanford University seeks applicants for a tenure track faculty appointment in the area of Evolutionary Developmental Biology at the rank of Assistant Professor. We seek applicants studying problems in the evolution of development, broadly defined to include work focused on understanding mechanisms of phenotypic evolution. Applicants are expected to develop a vigorous research program and to participate in both undergraduate and graduate education. For information about the Department consult **http://biology.stanford.edu/**.

Applicants should send an application containing: a cover letter (with email address and fax number), a curriculum vitae, names and email addresses of three references, a statement of research accomplishments and future plans, and a description of teaching experience to:

Chair, Evolutionary Developmental Biology Search Committee
Department of Biological Sciences
371 Serra Mall
Stanford University
Stanford, CA 94305-5020

Applicants should request that their reference letters be sent directly to the above address. Materials should be received by **December 1, 2006**. The term of the appointment would begin September 1, 2007.

Stanford University is an Equal Opportunity, Affirmative Action Employer.

Microbiology Faculty Positions

Stony Brook University's Department of Molecular Genetics and Microbiology in the School of Medicine invites applications for TWO tenure-track faculty positions at the Assistant Professor level in the fields of Viral and Bacterial Pathogenesis. Successful candidates will be expected to establish a vigorous extramural research program, direct graduate student and postdoctoral research, and participate in Departmental teaching and administrative responsibilities. The Department of Molecular Genetics and Microbiology and the adjacent Center for Infectious Diseases provide a highly interactive scientific community with world-class research facilities including two BSL-3 laboratories. The Department has training grants to support graduate students and postdoctoral fellows. The School of Medicine and Stony Brook University maintain core facilities that include imaging, sequencing, animal/transgenic, cell sorting, proteomics, microarray, bioinformatics, monoclonal antibodies, and cell culture.

Faculty Search in area of Host Response to Viral Infection: Applicants must have a Ph.D. or M.D./Ph.D. and have at least two years of postdoctoral experience. Outstanding candidates whose research is in the area of host response to viral infection are encouraged to apply. Special consideration will be given to candidates whose expertise will contribute to the study of human infectious disease. Specific areas of interest include, but are not limited to, RNA and DNA virology, pathogenesis, and immunity.

Faculty Search in area of Host Response to Bacterial Infection: Applicants must hold a Ph.D. or M.D./Ph.D. and have at least two years of postdoctoral experience. Outstanding candidates working in all areas of bacterial pathogenesis are encouraged to apply. The Department is particularly interested in candidates studying host responses to bacterial infection and whose research complements existing areas of expertise within the Department.

The review of applications for both positions will begin October, 2006, and will continue until December 31, 2006, or until the positions are filled.

To apply, please send a CV, a brief summary of accomplishments, future research interests (four pages total), and the names and contact information of three references to the search committee for the position you wish to apply for:

Nancy C. Reich, Ph.D., Chair of Viral Search Committee, Department of Molecular Genetics and Microbiology 130 Life Sciences Building, Stony Brook University, SUNY, Stony Brook, NY 11794-5222

David G. Thanassi, Ph.D., Chair of Bacterial Search Committee, Department of Molecular Genetics and Microbiology, 130 Life Sciences Building, Stony Brook University, SUNY, Stony Brook, NY 11794-5222

Equal Opportunity/Affirmative Action Employer. Women, people of color, individuals with disabilities, and veterans are encouraged to apply. Visit *www.stonybrook.edu/cjo* for complete job description and other employment opportunities.

STONY BROOK

OHIO UNIVERSITY

Ohio University invites candidates for **two tenure-track faculty positions**, one in **biomedical engineering** and one in **biomedical sciences**. The biomedical engineer will be appointed to a department within The Fritz J. and Dolores H. Russ College of Engineering (Chemical and Biomolecular, Mechanical, or Electrical Engineering and Computer Science). The biomedical scientist will be appointed to The Department of Biomedical Sciences in The College of Osteopathic Medicine (COM). Both candidates' research should address the cellular and molecular mechanisms of disease and/or focus on the identification and development of novel biomedical therapeutics (which includes drug delivery) or diagnostics. For the COM position we are particularly interested in candidates with expertise in immunology/innate immunity and the relevance of either to disease expression, e.g., cancer, infectious diseases, diabetes and its complications. The successful candidates will help to build the new Master's degree program in Biomedical Engineering and be expected to develop a dynamic externally funded research program. For the engineering position, applicants must have a PhD in biomedical engineering or a closely related discipline. The COM position is open to applicants with PhD, DO, or MD degrees. Post-doctoral research experience is required for both positions. Salaries will be based on qualifications.

Applications must be submitted online at **www.ohiouniversityjobs.com/applicants/Central?quickFind=51988** for the biosciences position and **www.ohiouniversityjobs.com/applicants/Central?quickFind=51995** for the biomedical engineering position. Review of applications will begin on December 1, 2006 and continue until positions are filled. Attach a curriculum vitae including contact information for 3 personal references, a statement of teaching interests and a statement of research interests. Questions regarding the positions can be directed to either **Dr. Douglas J. Goetz (goetzd@ohio.edu)**, chair Biomedical Engineering search committee, or **Dr. Leonard D. Kohn (lkohn1@rrohio.com)**, chair Biomedical Sciences search committee. We seek candidates with a commitment to working effectively with students, faculty and staff from diverse backgrounds.

Ohio University, located in a picturesque college town in rural southeastern Ohio, is an Affirmative Action, Equal Opportunity Employer with a Dual Career Network (http://www.ohio.edu/dual).

POSITIONS OPEN

ASSISTANT DIRECTOR, FLOW CYTOMETRY AND CELL SORTING CORE

Applications are invited for Assistant Director, Flow Cytometry and Cell Sorting (FACS) Core Facility in the University of South Carolina School of Medicine Instrumentation Resource Facility **(website: http://dba.med.sc.edu/price/irf/irf.htm).** This will be a full-time research track faculty position that will involve 50 percent time towards development of FACS core and 50 percent time dedicated to development of an individual's own research program. The FACS Core will be a service facility developed to assist other Principal Investigators and their laboratories in experiments that involve analysis and cell-sorting technologies. A strong background in immunology, experience in hands-on operation of a FACS system, and interpretation of FACS data are essential. A background in stem cell biology is desirable. The candidates can have research experience in any area of biomedical sciences although preference will be given to those interested in cancer, HIV/AIDS, or cardiovascular development and disease. Review of applicants will begin immediately and continue until the position is filled. Applicants should submit their curriculum vitae, future research plans, and three letters of reference to: **Dr. Robert Price, Director, Instrumentation Resource Facility, University of South Carolina School of Medicine, Columbia, SC 29208. E-mail: price@med.sc.edu.** *The University of South Carolina is an Affirmative Action, Equal Opportunity Institution.*

PROFESSOR OF MEDICINE AT BRIGHAM AND WOMEN'S HOSPITAL
Harvard Medical School

The Brigham and Women's Hospital and Harvard Medical School seek a Professor of Medicine to serve full-time, investigator criteria, in the Channing Laboratory and the Infectious Diseases Division, Department of Medicine, at Brigham and Women's Hospital. The Department seeks a distinguished Physician with strong expertise in the area of bacterial pathogenesis. Candidates should have an outstanding record of accomplishment in basic research, and a major commitment to medical students, residents, and fellows, experience in administration of an academic program as well as a strong commitment to all of the clinical, educational, and scientific activities of the Department. Academic credentials should be of sufficient strength to warrant an appointment as Professor at Harvard Medical School.

Interested individuals should send a letter of application and current curriculum vitae by November 1, 2006, to: **Dennis L. Kasper, M.D., Chairperson, Search Committee, Channing Laboratory, 181 Longwood Avenue, Boston, MA 02115.**

Brigham and Women's Hospital and Harvard Medical School are Equal Opportunity/Affirmative Action Employers. Women and minorities are particularly encouranged to apply.

ASSISTANT PROFESSOR

MIT's Department of Brain and Cognitive Sciences anticipates making a faculty appointment at the Assistant Professor level in cognitive science or cognitive neuroscience. Applicants should be conducting cognitive science or cognitive neuroscience research with humans in the areas of perception, learning, memory, attention, motor control, language, knowledge representation, reasoning, decision-making, social cognition, development, or computational modeling of cognition. It is important for applicants to identify the area or areas for which they are applying. Please enclose curriculum vitae, a statement of research and teaching interests, and representative reprints. In addition, please arrange to have three letters of recommendation sent to the **Search Committee.** Review of applications will begin as they are received. Send applications to: **Cognitive Search Committee, 46-2005, MIT, 77 Massachusetts Avenue, Cambridge, MA 02139-4307.** Information about the Department can be found at **website: http://web.mit.edu/bcs/.** *Qualified women and minority candidates are especially encouraged to apply. MIT is an Affirmative Action/Equal Opportunity Employer.*

POSITIONS OPEN

A **POSTDOCTORAL RESEARCH POSITION** is available immediately in the laboratory of **Dr. Mohamed Trebak,** Center for Cardiovascular Sciences at the Albany Medical College, Albany, New York. Topics of research focus on endothelial cell signaling, specifically, the role of transient receptor potential (TRP) ion channels in calcium entry pathways controlling proliferation and angiogenesis. We use a wide range of technologies including patch clamp electrophysiology, calcium imaging, molecular and cellular biology, and biochemical techniques.

The successful candidate will have a Ph.D. degree and proven research ability evidenced by publications in peer-reviewed journals with strong knowledge base and laboratory research experience in any of the following areas: biochemistry, cell biology, signal transduction, molecular biology. Special attention will be given to candidates with expertise, or the desire to obtain training, in the field of calcium imaging and patch clamp electrophysiology.

Interested individuals should send a cover letter and curriculum vitae and provide contact information of three references, preferably by e-mail to: **Jo Anne La Plante, Center for Cardiovascular Sciences (MC8), Albany Medical College; 47 New Scotland Avenue, Albany, NY 12208-3479. E-mail: laplanj@mail.amc.edu.** *Albany Medical Center is an Equal Opportunity Employer.*

CATEGORY: INDUSTRY POSITION RESEARCH SCIENTIST OR RESEARCH ENGINEER
Hitachi Chemical Research Center, Incorporated

Hitachi Chemical Research Center, Incorporated (HCR), a subsidiary of Hitachi Chemical Company, Limited, is located in southern California on the University of California, Irvine campus. HCR is a research and development company directed towards novel technology platforms and related biomaterials for life sciences. The candidate will focus on research in the nanotechnology or nanomaterial fields to develop polymer-based functional materials. The individual will create, develop and direct his/her own project. Candidate must be an independent researcher who has demonstrated scientific creativity and technical proficiency in his/her field. In addition, the candidate must have a strong background in a wide range of chemistry including biochemistry, preferably working with polymers or in material sciences. Position requires a minimum of a Ph.D. in appropriate science or engineering. HCR offers competitive benefits and salary. Interested candidates can e-mail resumes to **Ms. Lisa Osborn** at **e-mail: losborn@hcrcenter.com.** *Equal Opportunity Employer.*

MOLECULAR AND BEHAVIORAL NEUROSCIENCE

We invite applications for a tenure-track **ASSISTANT PROFESSOR** position to investigate the genetic and neurobiological basis of behavior. Any areas of behavior are of interest, such as learning, behavioral plasticity, sex differences, environmental interactions, or addictive behaviors. Preference will be given to candidates using genomic, proteomic, bioinformatic, or quantitative approaches. The successful candidate is expected to develop a vigorous, extramurally funded research program and participate in undergraduate and graduate instruction. The startup package includes a competitive salary, a generous budget, and an outstanding collegial environment. Send curriculum vitae, research statement, and three reference letters to **Doug Harrison** at **e-mail: dough@uky.edu** or **Department of Biology, University of Kentucky, 101 Morgan Building, Lexington, KY 40506** (see **website: http://www.as.uky.edu/biology**). Consideration of applications will begin December 1, 2006. *Equal Opportunity/Affirmative Action Employer. Women and minorities encouraged to apply.*

POSITIONS OPEN

FACULTY POSITION
Neurobiology

The Department of Neurobiology at the University of Pittsburgh School of Medicine is inviting applications for two full-time Tenure-Track positions, one in developmental neuroscience and the other in cellular and molecular neuroscience. For one of these positions we hope to identify researchers using genetically tractable vertebrate model systems, particularly zebrafish, to investigate fundamental questions in neurobiology. The position requires a Ph.D. and/or M.D. with a minimum of two years of postdoctoral experience. Applicants at all levels will be considered. Highly competitive salary support and startup funds will be made available. Applicants should submit by December 31, 2006, curriculum vitae, a detailed description of future research goals, representative recent publications, and names and addresses of three references by e-mail to **e-mail: recruit@neurobio.pitt.edu** or by mail to: **Dr. Susan G. Amara, Chair, Department of Neurobiology, University of Pittsburgh School of Medicine, 6th Floor Room 6057, Biomedical Science Tower 3, 3501 Fifth Avenue, Pittsburgh, PA, 15261, telephone: 412-383-8910. Website: http://www.neurobio.pitt.edu.**

The University of Pittsburgh is an Affirmative Action, Equal Opportunity Employer.

THE SARAH AND DANIEL HRDY VISITING FELLOWSHIP IN CONSERVATION BIOLOGY

The Department of Organismic and Evolutionary Biology invites both nominations and direct applications for the Hrdy Visiting Fellowship in Conservation Biology for the academic year 2007-2008. The Hrdy Visiting Fellowship is available either at the **SENIOR FACULTY** level or at the **JUNIOR (i.e., POSTDOCTORAL)** level for one or two semesters. Duties will include teaching one course and/or giving lectures in conservation biology, as well as research and collaboration with members of the Harvard community. Applicants should contact a faculty sponsor(s), with whom they will collaborate, before applying. Please send a cover letter with a statement of intent, along with curriculum vitae and representative publications, and arrange to have three letters of reference sent to:

Committee for Hrdy Fellowship in Conservation Biology
Department of Organismic and Evolutionary Biology
26 Oxford Street
Cambridge, MA 02138

Review of applications will begin on November 15, 2006. *Harvard University is an Equal Opportunity Employer.*

TENURE-TRACK FACULTY POSITION
Division of Infectious Diseases at the University of Pittsburgh

The Division of Infectious Diseases at the University of Pittsburgh invites applications for a tenure-track faculty position at the **ASSISTANT/ASSOCIATE PROFESSOR** level. A scientist is sought with an interest in antiviral drug discovery and/or antiviral drug resistance in HIV, hepatitis B, hepatitis C, or influenza to work in a dynamic research environment. Qualified applicants must hold a Ph.D and/or M.D. degree and should have an established research program or be able to develop such a program within two years. Startup resources and compensation are available commensurate with experience. Applicants should submit a cover letter, curriculum vitae, statement of research interests, and the names and contact information of three qualified references to: **Nicolas Sluis-Cremer, Ph.D., Division of Infectious Diseases, University of Pittsburgh, S817 Scaife Hall, 3550 Terrace Street, Pittsburgh, PA 15261; e-mail: cremern@dom.pitt.edu.** Applications will be reviewed as they are received and will be accepted until the position is filled.

FACULTY POSITION

Professor of Medicine in Quantitative Systems Biology

Brigham & Women's Hospital Harvard Medical School

The Genetics Division in the Department of Medicine at Brigham & Women's Hospital and Harvard Medical School seeks an outstanding Professor level scientist in the area of systems biology. Candidates for the rank of Associate Professor will also be considered. Areas of interest include analysis of the behavior of complex genetic, metabolic and developmental networks, and the development of mathematical models for the same. Importance will be attached to the applicant's desire and ability to work in a highly inter-disciplinary, interactive, medically and biologically directed environment. Applicants should possess PhD, MD, or MD, PhD degrees and have an established national or international reputation. The successful applicant will enjoy outstanding resources and state-of-the-art facilities, and will be able to interface with strong institutional expertise in numerous areas of medicine, biology and physical science.

Send CV, description of research interests and names of three references by **December 31, 2006** to:

Richard Maas, M.D., Ph.D., Chair,
Search Committee
BWH Genetics Division
New Research Bldg., NRB 458H
Harvard Medical School
77 Avenue Louis Pasteur
Boston, MA 02115

BWH and HMS are Equal Opportunity Employers.

Professor and Chair Department of Biochemistry University of Nebraska-Lincoln

The University of Nebraska-Lincoln is seeking an individual with an outstanding research program and excellent interpersonal skills who can provide energetic and creative leadership for the research, teaching, and public service activities of its **Department of Biochemistry**. A competitive start-up package is available for this full time, 12-month appointment. The **Department of Biochemistry** is rapidly growing and includes 15 budgeted and 10 affiliated faculty members. The research programs in the **Department** are currently supported by annual grant and contract awards exceeding $5 million. The **Department** houses the NIH-funded Redox Biology Center and has established strengths in biomedical research, plant biochemistry, structural biology, bioinformatics, and classical enzymology. The **Department** is located in the state-of-the-art George W. Beadle Center, which is also the home of the NIH-funded Nebraska Center for Virology, the Plant Science Initiative, the Center for Biotechnology, and key core research facilities. To learn more about the **Department**, please visit the website **http://biochem.unl.edu**.

To apply for this position, access the web site **http://employment.unl.edu**. Search for position number **060852**. Complete the faculty academic administrative information form. Attach a letter of application, curriculum vitae, and the contact information for three professional references. Review of applications will begin on **November 16, 2006**, and continue until the position is filled.

The University of Nebraska is committed to a pluralistic campus community through Affirmative Action and Equal Opportunity and is responsive to the needs of dual career couples. We assure accommodation under the Americans with Disabilities Act; contact Linda Arnold at 402-472-3802 for assistance.

Stanford University Molecular/Cell Biologist Faculty Position

The Department of Biological Sciences at Stanford University welcomes applicants for a tenure track faculty appointment at the Assistant Professor rank. We seek individuals studying basic problems in molecular biology and cell biology, at any level from molecules to tissue organization. We are particularly interested in applicants addressing these problems using state-of-the-art methods in biological imaging, proteomics and metabolomics, and/or systems biology. Applicants are expected to develop a vigorous research program and to participate in both undergraduate and graduate education. For information about the Department consult **http://biology.stanford.edu/**.

Applicants should send an application containing: a cover letter (with email address and fax number), a curriculum vitae, a statement of research accomplishments and future plans, a description of teaching experience, and names and email addresses of three references to: **Chair, Molecular/Cell Search Committee, Dept. of Biological Sciences, 371 Serra Mall, Stanford University, Stanford, CA 94305-5020.** Applicants should request that their reference letters be sent directly to the above address. Materials should be received by **December 1, 2006**. The term of the appointment would begin September 1, 2007.

Stanford University is an Equal Opportunity, Affirmative Action Employer.

AWARDS

Harold M. Weintraub Graduate Student Awards – 2007

The Fred Hutchinson Cancer Center is seeking nominations for outstanding Graduate Students for the Harold M. Weintraub Graduate Student Award to recognize outstanding achievement during Graduate Studies in the Biological Sciences. Awardees will participate in a scientific symposium honoring Hal Weintraub and his commitment to innovative science.

The eighth annual Award Symposium will be held May 4-5, 2007. Graduate Student Awardees will be selected from among those nominated on the basis of quality, originality, and significance of their work, as well as to represent a diverse range of research topics. The Hutchinson Center Weintraub and Groudine Fund, established to foster intellectual exchange through the promotion of programs for graduate students, fellows and visiting scholars, will cover expenses for the Graduate Student Awardees.

One nomination may be submitted per Department or Program. The nomination should be submitted by the Department or Program Chairperson and include the student's CV, a one page description of the thesis work conducted, and a recommendation letter from the student's mentor. Additional information concerning the Award and nomination process can be found at the website listed below.

The nomination should be submitted ONLINE by **December 15, 2006** -- following the instructions at the website: **http://www.fhcrc.org/science/basic/weintraub/**.

Questions regarding this Award should be addressed to **Susan Parkhurst (susanp@fhcrc.org)**.

ETH

Eidgenössische Technische Hochschule Zürich
Swiss Federal Institute of Technology Zurich

Science City: International Sustainability Competition

ETH Zurich is pleased to announce the "International Sustainability Competition Science City". This is a global competition for interdisciplinary teams to provide ideas for how Science City can realize its vision of a new model for the sustainable integration of science and society. Teams from academic institutions are particularly encouraged to participate. Entrants will be asked to combine competencies from different fields, for example spatial planning, urban development, mobility, sociology, arts and culture, economics and management, and must demonstrate sound knowledge and experience in sustainability-related topics.

With Science City, ETH Zurich establishes a university campus where science and society can meet and which serves as a model for sustainability. Science City, located at Hoenggerberg near the center of Zurich, is much more than a conventional university campus. Science City embodies the vision of an urban neighborhood where people not only teach, study and conduct research, but where they also live, shop, socialize, exercise, attend cultural events and much more. It represents a culture of thinking and dialogue.

Information:
www.sciencecity.ethz.ch/internationalcompetition

Organisation/Contact:
Novatlantis – Sustainability at the ETH Domain
Tanja Lütolf, Tel: +41 44 305 94 65, **luetolf@novatlantis.ch**

The Cystinosis Research Foundation 2006 Autumn Call for Funding Proposals

$1.2 Million Available

The Cystinosis Research Foundation's (CRF) ultimate goal is a cure for this disease. Research awards will be given for up to two years. The CRF has over $1.2 million dollars in research funds available. The number of awards and their value will depend on the number of outstanding proposals and the funds available at the time.

- Research Proposal — The CRF is pleased to announce its second 2006 call for research proposals. The CRF is prepared to fund proposals to improve the immediate care of children and young adults with cystinosis and to develop new understanding and treatment of cystinosis to help these children.
- Post-Doctoral Fellowships — The CRF plans to establish the first post-doctoral research fellowship program in the United States to encourage young investigators to establish careers in cystinosis research. Fellows will be funded for 2-3 years to a maximum of $75,000/year.

For the current funding cycle, proposals and fellowship applications must be received by November 6, 2006. Decisions for funding will be made by the end of the year. **For instructions on how to prepare proposals, visit www.natalieswish.org and click on research/grant guidelines.**

Review Process — Proposals are reviewed by a Scientific Review Board comprised of experts on Cystinosis who then advise the CRF on the scientific merit of each proposal. The CRF will balance the eventual funding to support clinical and bench research, and fellowships.

AWARDS

Letters of Intent are Being Accepted for

Alzheimer's Disease Research Centennial Awards

$1,000,000 each

- Seeking innovative, multi-disciplinary projects.
- Open to basic science and/or clinical research collaborations.
- Available to domestic and international researchers.
- Research plan must be a collaborative project with one principal investigator running own lab and include two additional labs not from the same institution.
- Letters of Intent due Tuesday, November 21, 2006. Invited applicants, notified in mid-December, will submit full application.
- Two grants at $1,000,000 each (payable over two years) will be awarded.
- Grants awarded on March 31, 2007.

Further details and information available at **www.ahaf.org**; Email: **grants@ahaf.org** or phone: **301-948-3244**.

ALZHEIMER'S DISEASE RESEARCH
A Program of the
American Health Assistance Foundation

WORKSHOPS

Workshop: Functional Genomics of Malaria Parasites

March 18–27, 2007, in Pathumthani, Thailand

National Center for Genetic Engineering and Biotechnology (BIOTEC)

The course will equip participants with the expertise to construct transgenic *Plasmodium falciparum* and *P. berghei* parasites and to apply this knowledge in their home laboratories. The workshop is limited to approximately 20 places. Scientists from malaria endemic countries are encouraged to apply.

REQUIREMENTS: Ph.D. students or postdoctoral scientists with previous experience in molecular techniques and a thorough understanding of molecular and cell biology. Submit applications to biotecworkshop@wehi.edu.au by **November 15, 2006**.

COURSE TOPICS: Malaria parasites and their genomes • Cultivation, transfection, and selection of *P. falciparum* and *P. berghei* • Vector design and construction • Drug resistance and visual markers • Analysis of transgenic parasites • New technologies

ORGANIZERS: Alan Cowman & Brendan Crabb, chairs (WEHI, Australia); Sumalee Kamchonwongpaisan & Chairat Uthaipibull (BIOTEC, Thailand); Andy Waters (LUMC, The Netherlands)

Application and more information:
www.wehi.edu.au/BIOTECworkshop

Sponsors: Howard Hughes Medical Institute; National Center for Genetic Engineering and Biotechnology; The Walter and Eliza Hall Institute; Special Programme for Research and Training in Tropical Diseases (TDR) of the United Nations Development Programme/World Bank/World Health Organization; and BioMalPar, supported by the European Commission as part of the Sixth Framework Programme (FP6)

PRIZES

2007 Novartis Immunology Prizes

The 2007 Novartis Prizes, one for Basic Immunology and one for Clinical Immunology are awarded for outstanding achievements in the understanding of immunology and major immunological discoveries that lead to therapeutic applications in such fields as transplantation, haematopoiesis, cancer immunology, immunity to infectious diseases, rheumatology, dermatology and asthma. For more information, please visit www.novartisimmunologyprizes.org

The next Novartis Immunology Prizes will be awarded at the XIIIth International Congress of Immunology in Rio de Janeiro on 23 August 2007 in a special Award Ceremony during Thursday afternoon.

Each prize is worth SFr 100 000

Nominations in English should comprise a summary of the research work up to 2 pages, a curriculum vitae, a bibliography and reprints of not more than 3 key published papers in English or with extended summaries in English.

The deadline for entries is 31 January 2007.

They should be sent, including an electronic version of the summary, to:
Dr Erik Wiskott, Novartis Prizes for Immunology, P.O. Box 360, CH 4013, Basel, Switzerland. Fax: +41 61 421 9019 email: erik.wiskott@novartis.com

Judges: Andrew McMichael (Chair), Jean-François Bach, Max D. Cooper, Tasuku Honjo, Philippa Marrack, Randall Morris, Hidde Ploegh, David H. Sachs, Jan de Vries.

NOVARTIS

CONFERENCE

IBRO

7th IBRO World Congress of Neuroscience

Melbourne, Australia

Melbourne Exhibition and Convention Centre

12-17 July 2007

Deadline for abstract submission 31st January 2007

Full program at www.ibro2007.org

EIGHT Plenary Lectures
Peter Agre
Norio Akaike / Lily Jan / Herta Flor
Edvard Moser / Simon Gandevia
Mu-Ming Poo / Mandyam Srinivasan

SIXTY FOUR Symposia
Workshops / Posters / Social events
Plus:
SEVENTEEN Satellite meetings

FIVE DAYS of Top-class Brain Science Down Under!

Organising Committee Secretary: A.Lawrence@hfi.unimelb.edu.au
Conference Secretariat: ibro2007@sallyjayconferences.com.au

POSITIONS OPEN

CURATOR/DIVISION OF INSECTS
Field Museum of Natural History

The Department of Zoology of the Field Museum seeks a scientist with research programs focused on arthropods to fill a career-track appointment at the **ASSISTANT CURATOR** level. Candidates should have a Ph.D. and a proven record of scientific achievement in collections-based research. Beyond taxonomic focus, we are searching broadly for excellence in evolutionary biology in such areas as phylogenetics, comparative morphology, molecular biology, development, biogeography, coevolution, and conservation.

In addition to research, responsibilities include curation of globally important collections in the Division of Insects, participation in public exhibit and education programs, and administration. Strong relationships with local Universities provide opportunities for participation in graduate and undergraduate training and teaching. Applications should include: (1) curriculum vitae; (2) a statement of research and curatorial interests; (3) names and contact information of three referees; and (4) copies of up to five relevant publications. Review of applications will begin January 1, 2007. Send materials to: **Search Committee, Department of Zoology, Field Museum, 1400 South Lake Shore Drive, Chicago, IL 60605-2496.** Application materials as e-mail attachments preferred (receipt will be acknowledged). **E-mail: zoologyarthropods@fieldmuseum.org.** The Field Museum's homepage is **website: http://www.fieldmuseum.org.** The Departmental website is **website: http://www.fieldmuseum.org/research_collections/zoology/default.htm.** *The Field Museum is an Equal Opportunity Employer, and encourages applications from women and minorities.*

BACTERIAL PATHOGENESIS

POSTDOCTORAL FELLOW position is available immediately to join the collaborative research groups of **Drs. David N. McMurray and Jeffrey D. Cirillo** studying tuberculosis pathogenesis. Selected individual will be primarily responsible for conducting independent research on mycobacterial pathogens and publication of results. Research will emphasize the molecular, cell biological, and immunological characterization of virulence determinants in mycobacteria and their interactions with the host in a guinea pig virulence model. Ph.D. required and a record of productive experience in molecular biology of bacterial pathogens preferred. Send curriculum vitae and names and addresses of three references postmarked by October 31, 2006 (or until a suitable candidate is found), to: **Dr. Jeffrey D. Cirillo, Department Microbial and Molecular Pathogenesis, Texas A&M University Health Science Center, M.S. 1114, 471 Reynolds Medical Building, College Station, TX 77843-1114. Fax: 979-845-3479; e-mail: jdcirillo@medicine.tamhsc.edu.** Contact **Dr. Cirillo, telephone: 979-458-0778** for additional information.

Texas A&M University System Health Science Center is an Affirmative Action/Equal Opportunity Employer and encourages applications from women and minorities.

POSTDOCTORAL RESEARCH POSITIONS
Center for the Physics of Information
California Institute of Technology

The Center for the Physics of Information at the California Institute of Technology will have Postdoctoral Scholar positions available beginning in September 2007. Researchers interested in all aspects of the interface between information science and physical science are invited to apply.

Please apply online at **website: http://www.ist.caltech.edu/joinus/positions.html.**

Electronic copies of your curriculum vitae, publication list, statement of research interests, and three letters of recommendation are required.

The deadline for receipt of all application materials is January 12, 2007.

The California Institute of Technology is an Equal Opportunity/Affirmative Action Employer. Women, minorities, veterans, and disabled persons are encouraged to apply.

POSITIONS OPEN

INSTITUT PASTEUR

POSTDOCTORAL FELLOWSHIPS
Institut Pasteur, Paris, France

Founded in 1887 by Louis Pasteur and located in the heart of Paris, the Institut Pasteur is a world-renowned private research organization. The Pasteur Foundation is seeking outstanding Fellowship Applicants. Candidates may apply to any laboratory within 10 departments: cell biology and infection; developmental biology; genomes and genetics; immunology; infection and epidemiology; microbiology; neuroscience; parasitology and mycology; structural biology and chemistry; and virology. See website for details.

Fellowships are $60,000 per year for three years ($45,000 stipend plus $15,000). *U.S. citizenship required.* Deadline: February 2, 2007.

E-mail: pasteurus@aol.com.

Website: http://www.pasteurfoundation.org.

POSTDOCTORAL RESEARCH POSITIONS
Institute for Quantum Information
California Institute of Technology

The Institute for Quantum Information at the California Institute of Technology will have Postdoctoral Scholar Positions available beginning in September 2007. Researchers interested in all aspects of quantum information science are invited to apply.

Please apply online at **website: http://www.iqi.caltech.edu.** Electronic copies of your curriculum vitae, publication list, statement of research interests, and three letters of recommendation are required. The deadline for receipt of all application materials is January 12, 2007.

The California Institute of Technology is an Equal Opportunity/Affirmative Action Employer. Women, minorities, veterans, and disabled persons are encouraged to apply.

A **POSTDOCTORAL POSITION** is available immediately to study the molecular mechanism of pancreatic beta cell adaptation and development on transgenic/diabetic animal models. Applicants must have a Ph.D. degree with experience in molecular biology, biochemistry, metabolism, immunohistochemistry, cell culture, insulin secretion, and animal surgery. Send curriculum vitae and three references to: **Dr. Ye Qi Liu, Kosair Children's Hospital Research Institute, Department of Pediatrics, University of Louisville, 570 S. Preston Street, Suite 304, Louisville, KY 40202. Telephone: 502-852-2654, e-mail: yqliu001@gwise.louisville.edu.**

POSTDOCTORAL FELLOW

UNIVERSITY OF CINCINNATI (UC), COLLEGE OF MEDICINE, GENOME SCIENCE. Research in molecular biology, biochemistry, and cell culture in animal models. Specifically, research on signaling mechanisms regulated by atypical protein kinase Cs and their adapters and regulators. A Ph.D. is required. Pay is commensurate with experience. For additional information and to apply for position (26UC3420), please see **website: http//:www.jobsatuc.com.** *UC is an Affirmative Action/Equal Opportunity Employer.*

POSTDOCTORAL FELLOW to work on neural stem cells derived from human embryonic stem cells. Must have molecular biology background with skill in cloning and engineering genes. The ultimate goal is to design neural stem cells that can be used for transplantation.

Please send curriculum vitae and two letters of recommendation online to **e-mail: lweiner@usc.edu.**

Leslie P. Weiner, M.D., Department of Neurology, Keck School of Medicine of the University of Southern California.

POSITIONS OPEN

POSTDOCTORAL POSITION

An NIH-supported position is available to study protein interaction networks. A project to map interactions among all of the *Drosophila* proteins has thus far generated an interaction map involving proteins encoded by half of the fly genes. The Postdoctoral position is available to continue this project, to analyze gene and protein interaction networks, and to help develop technologies for biological validation of these networks. The laboratory is well equipped for high throughput assays and analyses, with state-of-the-art robotics and computer facilities. Other members of the group are studying cell cycle regulatory networks, validation of protein networks by RNAi knockdown in cultured cells, and molecular genetic analyses in *Drosophila*. For more information go to **website: http://proteome.wayne.edu.** Send curriculum vitae to: **Dr. Russ Finley, Center for Molecular Medicine and Genetics, Wayne State University School of Medicine, 540 East Canfield Avenue, Detroit, MI 48201, e-mail: rfinley@wayne.edu.**

FELLOWSHIPS

BULLARD FELLOWSHIPS IN FOREST RESEARCH
Harvard University

Each year Harvard University awards a limited number of Bullard Fellowships to individuals in biological, social, physical, and political sciences to promote advanced study, research, or integration of subjects pertaining to forested ecosystems. The Fellowships, which include stipends up to $40,000, are intended to provide individuals in midcareer with an opportunity to utilize the resources and to interact with personnel in any department within Harvard University in order to develop their own scientific and professional growth. In recent years Bullard Fellows have been associated with the Harvard Forest, Department of Organismic and Evolutionary Biology, and the J. F. Kennedy School of Government and have worked in areas of ecology, forest management, policy, and conservation. Fellowships are available for periods ranging from six months to one year after September 1 annually. Applications from international scientists, women, and minorities are encouraged. Fellowships are not intended for graduate students or recent postdoctoral candidates. Information and application instructions are available on the Harvard Forest **website: http://harvardforest.fas.harvard.edu.** Annual deadline for applications is February 1.

MARKETPLACE

PIERCE

The Protein People™

High-Performance Dialysis

Dialysis tube VS. cassette

SLIPPERY WHEN WET. Flat tubing is difficult to handle and fill when wet.	SAMPLE HANDLING	Easy to hold on to frame and inject sample.
SLIPPERY WHEN WET. Sample can easily be lost when tubing leaks or clamps slip off.	SAMPLE RECOVERY	>95% sample recovery.
SLIPPERY WHEN WET. Leaking into dialysate can compromise sample.	SAMPLE INTEGRITY	Sample remains intact with no contamination from surrounding dialysate.
SLIPPERY WHEN WET. Typically dialyze overnight. Difficult to recover sample from wet tubing.	SPEED	High surface area/sample volume ratio will dialyze twice as fast as conventional tubing.

No more leaking! Avoid sample loss with Pierce Slide-A-Lyzer® Dialysis Cassettes.

The Pierce Slide-A-Lyzer® Dialysis Cassettes have a silicone-like gasket that prevents them from leaking. The new color-coded transparent frames allow you to instantly know the MWCO of the membrane and to see the sample being injected. No knots, caps, lids or clamps to loosen, fall off or leak. Obtain >95% sample recovery with a rigid frame that permits smooth and complete withdrawal of samples.

FREE picture frame offer!*

Buy any package of Slide-A-Lyzer® Dialysis Cassettes before 12/31/06 and receive a free magnetic picture frame that looks just like our Cassettes!

How does the Cassette dialyze?

1. Inject sample into the Cassette through one of the guide ports on top of the gasket. Draw up on the syringe to remove air.
2. For Cassettes other than the 12-30 ml unit, attach a flotation buoy and dialyze. Forget about having to hassle with dialysis bag suspension!
3. After dialysis, inject the Cassette chamber with air, and withdraw your dialyzed sample from the Cassette.

www.piercenet.com

PIERCE

20 K MWCO

Slide-A-Lyzer® Cassettes are available in 2, 3.5, 7, 10 and now 20 K MWCO. See our website for the full line that best serves your dialysis needs.

ISO 9001 REGISTERED

Tel: 815-968-0747 or 800-874-3723 • Fax: 815-968-7316
Customer Assistance E-mail: CS@piercenet.com

For distributors outside the U.S. and Europe, visit www.piercenet.com

For European offices and distributors, visit www.perbio.com

PERBIO